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RESEARCH AND DEVELOPMENT OF HUMAN AND PRIMATE ANTIBODIES FOR IMMUNOTHERAPY OF VIRAL HEMORRHAGIC FEVER INFECTIONS

Final Report

Richard M. Condie

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The following report describes the methods used to produce human and primate immune globulin to Lassa fever and Argentinian hemorrhagic fever viruses and the results of the United States Army Medical Research Institute of Infectious Diseases (USAMRIID) testing of these products. Each preparation has viral neutralizing activity and is a safe, stable, well tolerated solution suitable for intravenous injection. Included also are the experiments to demonstrate the ability of the above methods to remove Human Immunodeficiency Virus (HIV) from the raw plasma.

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SUMMARY

During the expanse of time from 8/30/84 to 12/31/88 work was ongoing for the development of methods to produce safe and effective human and primate antibodies for immunotherapy of viral hemorrhagic fever infections. Contract Number DAMD 17-84-C-4046 was extended and continued with Contract Number DAMD 17-85-C-5210 which also included experiments to demonstrate the efficiency of our methods to remove Human Immunodeficiency Virus (HIV) from the plasma. For ease of reporting, the experiments have been separated into three groups each of which encompass the materials, methods, results and discussion and are designated as follows: Part I Lassa Fever, Part II Argentinian Hemorrhagic Fever and Part III HIV Spiking Experiments.

Due to the rarity and short supply of human convalescent plasma, two approaches were utilized to provide a suitable material for prevention and treatment of these infections in man. First, conversion of low titer non-protective human convalescent plasma to an effective intravenous IgG preparation and second, development of an effective monkey IgG preparation from high titer protective monkey plasma. These approaches expand the potential for raw plasma sources for the rare materials as well as providing a means for stabilizing the material for long-term storage and simplifying the logistics for distribution.

From plasma pools supplied by the United States Army Medical Research Institute of Infectious Diseases (USAMRIID), there were five different human anti-Lassa fever (ALF) preparations, two monkey anti-Lassa fever (ALF) preparations and three human Argentinian anti-hemorrhagic fever (AHF) preparations completed for this project. The final products include: 1) Human ALF IgG containing all four subclasses IgG_1, IgG_2, IgG_3, IgG_4; 2) Human ALF IgG containing only the three subclasses IgG_1, IgG_2 and IgG_4; 3) Human ALF $F(ab')_2$; 4) Human ALF IgG from Cohn fractionation; 5) Human ALF IgG from Cohn fractionation which was also pepsin deaggregated; 6) Monkey ALF IgG; 7) Monkey ALF $F(ab')_2$; 8) Human AHF IgG containing all four subclasses IgG_1, IgG_2, IgG_3, IgG_4; 9) Human AHF containing only the three subclasses IgG_1, IgG_2 and IgG_4; and 10) Human AHF $F(ab')_2$.

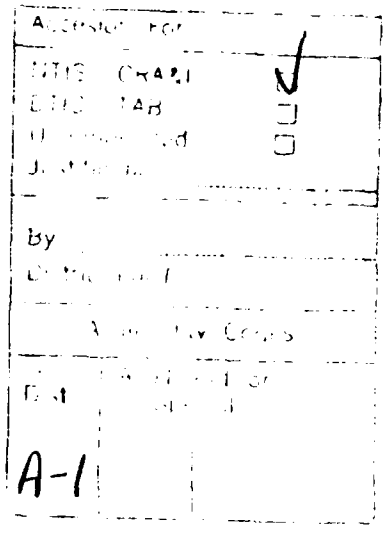
In order to ensure that the final product preparations were safe for intravenous use, free from HIV, experiments were completed to assess the ability of each step in the fractionation process to remove and/or inactivate HIV. In eight experiments the starting material was normal human plasma (supplied by Minnesota ALG) and two experiments started with HTLV-III antibody positive human plasma (supplied by California Department of Health). Each experiment was spiked at some point with live HTLV-III and resulting infectivity was assessed by measuring the cytopathic effect on MT-2 cells (California Department of Health, Berkeley, CA).

The results of the testing by USAMRIID showed that all of the human intact IgG ALF and AHF preparations had protective titer as demonstrated in the Log_{10} Neutralizing Index (LNI) and all proved effective in protecting guinea pigs against viral challenge. There were only marginal differences between the various types of preparations at the 3 ml/kg dose. The $F(ab')_2$ showed very little protective activity and was considerably less effective than the unfractionated immune plasma.

In the human plasma HIV experiments, the results indicated a reduction in viral infectivity at each step of our process. Most importantly, the final step of

QAE column chromatography in each experiment was capable of removing all of the activity even when additional virus was spiked immediately prior to the column.

These experiments have shown that it is possible to utilize low titer non-protective human convalescent plasma to produce an effective intravenous AHF or ALF IgG. Several choices are available for a preparation method for producing large quantities of an immune globulin with high neutralizing activity which is also stable, well tolerated, safe and virus free.



FOREWORD

In conducting the research described in this report, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

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I. Introduction

A. Type of Project:

This study completed Contract Number DAMD 17-84-C-4046 work on the preparation of effective, intact and fragmented $F(ab')_2$ primate and human immune globulin to Lassa fever virus and includes work on Contract Number DAMD 17-85-C-5210 to produce human immune globulin to Argentinian Hemorrhagic Fever virus. All of the plasma was supplied by USAMRIID; the Minnesota ALG Program (MALG), Department of Surgery, University of Minnesota, prepared the immunoglobulin fractions which were returned to USAMRIID for testing and evaluation. Also included is work on Contract Number DAMD 17-85-C-5210 to demonstrate the efficiency of the MALG fractionation process to remove and/or inactivate Human Immunodeficiency Virus.

B. Technical Objectives

The objectives of this project were the preparation of stable, safe, effective, intact IgG and $F(ab')_2$ fragments from high titer monkey and high and low titer human convalescent plasma to Lassa fever and Argentinian hemorrhagic fever viruses for the possible future use in the prevention and treatment of these infections in man. To develop and provide the necessary information for determining which of these approaches has validity in the clinical setting the following studies were completed:

1. Preparation of a safe and effective, native, intact, intravenous IgG from convalescent human hemorrhagic fever plasma.
2. Preparation of a native, intact IgG from monkey plasma with high neutralizing activity to Lassa fever virus.
3. Preparation of 99% pure $F(ab')_2$ fragments from convalescent human and high titer monkey plasma to Lassa fever and Argentinian hemorrhagic fever viruses and compare these fragments with intact IgG in prevention and treatment of Lassa and Argentinian hemorrhagic fever virus infections in animal models developed at USAMRIID.
4. Determination of the ability of the MALG procedures to remove or inactivate HIV.

C. Hypothesis

The immunity developed from experience with a number of viruses whether acquired either by vaccination or "natural" infection results from the integration of a number of host defense mechanisms involving humoral antibodies, complement, the phagocytic system and the cellular immune systems mediated by specific T-cell lymphocytes. The object of passive serum immunity is to administer an excess of specific viral neutralizing antibody in order to prevent, block, or favorably modify infection. This type of therapy has been used successfully in the prophylaxis of hepatitis, measles, and most recently by ourselves in the prevention of CMV infection in high risk bone marrow transplant recipients (1,2,28,29). In order for this to be most effective, it has been the general opinion that the unaltered, intact structure of the native immunoglobulin molecule was

required since combination with the virus was but the first step in a series of reactions involving complement fixation, lysis, and phagocytosis by the reticuloendothelial system. While the antibody combining site located in the Fab portion of the molecule is responsible for initial and specific interaction with the virus, once this interaction is completed, it is the Fc portion which determines complement fixation, lysis, phagocytosis and circulatory half-life. There are numerous reports from the 1960's that demonstrated Fab and $F(ab')_2$ fragments of viral neutralizing IgG had either lost neutralizing activity or were markedly reduced in activity (3,4,5). Therefore, loss of the Fc portion of the immunoglobulin molecule resulted in reduction of neutralizing activity. Also, there have been reports that non-neutralizing monoclonal antibody could prevent virus neutralization by blocking the attachment of neutralizing antibody to the critical site of the virus (6). These facts all suggest that for successful passive immunotherapy, the antibody should be an intact, native, IgG comprised of only neutralizing antibody (1,7). Recently, however, there have been a number of reports of virus infection in which antibodies appear to produce effects which are detrimental to the host. The phenomenon known as antibody dependent or antibody mediated enhancement of viral replication (26,27) has been implicated in early death in mice to rabies (8), enhancing viral replication in culture macrophages with West Nile virus (9), and in dengue virus infection in children (10).

Clinically, dengue virus normally produces a non-fatal illness with a rash, but a proportion of children in Southeast Asia develop hemorrhagic manifestation and may die from shock (10). Although the effector mechanism responsible for the shock remains unresolved, laboratory studies related to hemorrhagic dengue suggest antibody to be related to the early death phenomenon (11,12). Hallstead showed that monkeys innoculated with dengue virus mixed with sub-neutralizing concentrations of anti-dengue antibodies showed higher levels of viremia than control animals given dengue virus alone (11). Dengue virus replicates poorly in normal human or monkey peripheral blood white cell preparations, but shows enhanced yields of virus when exposed to infectious virus antibody mixtures (12). This antibody mediated enhancement of viral replication is not restricted to dengue virus, but also has been demonstrated in continuous cell lines of mouse and human macrophages with West Nile virus and other Togaviridae and Bunjaviridae (9). Hallstead first proposed that the mechanism involved in the human monocyte system depended on an increased uptake of viruses complexed with antibody through Fc receptors, the internalized virus escaping destruction by the phagocytes and replicating within the cell (12). Peiris showed than the antibody enhancement of West Nile virus replication was specifically blocked by monoclonal antibodies directed against Fc receptors on the cells they were using (13). To that point Hallstead also earlier showed that neutralizing anti-dengue $F(ab')_2$ antibody did not result in enhanced viral replication in human monocytes (12).

These observations raised several important and interesting questions relating to the type of immunoglobulin preparation that should be prepared for future clinical studies. First, since antibody mediated enhanced viral replication could well operate in Argentinian hemorrhagic fever, efforts should be addressed to circumvent the phenomenon with

neutralizing antibody lacking the Fc part of the molecule--an $F(ab')_2$ fragment prepared from convalescent Argentinian fever human plasma. Hallstead's work suggests that $F(ab')_2$ fragments of dengue fever antibody possess neutralizing activity. Second, should $F(ab')_2$ prove effective in viral neutralization, then high titer monkey $F(ab')_2$ antibody would be prepared and tested since these animals can be immunized to produce extremely high titer and effective antiviral antibodies. Third, is the antibody mediated enhanced viral replication due to low or sub-optimal levels of neutralizing activity or is there an "enhancing antibody"? If so, what are its IgG subclass distributions?

D. Background

1. Basis

Hemorrhagic fevers produced by arenaviruses (Argentinian, Bolivia, and Lassa fevers) are severe diseases associated with high mortality, for which no specific preventative or therapeutic measures are available (19). The mortality of experimental infections with arenaviruses can be reduced with immune plasma (16) and clinical observations suggest that administration of immune plasma early in the course of the disease may be useful (25). In fact, in a recent double blind trial with immune plasma obtained from Argentinian hemorrhagic fever (AHF) donors, convalescent plasma immunotherapy initiated within 8 days of the onset of the disease resulted in a much lower mortality than those given normal plasma (17). However, some patients treated with immune plasma developed late neurological complications. The association between treatment with immune plasma and late neurological sequellae suggests an immunological mechanism possibly related to the antibody mediated enhancement proposed for severe dengue hemorrhagic fever. While this treatment resulted in significantly lower mortality, the logistics of plasma quality, collection, dosage and timing suggest the need for a more predictable and reliable immunotherapy with specific hyperimmune antibody. Whether a neutralizing $F(ab')_2$ AHF antibody would be effective and not result in late neurological problems remains to be determined, but should be tried in monkey models where the same late neurological syndrome results (18).

Lassa virus infections of humans have been recognized since the first observation in 1969 of a severe, generalized disease syndrome described as Lassa fever in Nigeria (25). It is a major disease in Western Africa, particularly well documented in Nigeria, Sierra Leone and Liberia. The causative agent, Lassa virus, is disseminated from persistently infected rodent hosts, and is highly infectious by aerosol; it may be handled safely only with maximum containment laboratories. Nosocomial transmission has been responsible for focal outbreaks of Lassa fever in field hospitals; the virus could well pose a serious medical threat.

The mortality rate for hospitalized Lassa fever patients with symptomatic and supportive treatment approximates 20%. Specific treatment, using convalescent Lassa-immune plasma, is widely used, but

efficacy has never been systematically evaluated (14,15,25). Evaluation of plasma immunotherapy for Lassa fever like AHF has been plagued by difficult to control variables such as quantity, quality and timing of immune plasma administration.

Systematic evaluation of the immunotherapy concept for treatment of Lassa virus infection has been facilitated by Dr. Jahrling's (USAMRIID) development of a uniformly responding animal model and the establishment of biologically relevant criteria for immune plasma and immune IgG selection and administration.

Inbred guinea pigs and cynomolgus monkeys provide good models for lethal human Lassa fever and have proven to be useful for protective efficacy trials of immune plasma. Protective efficacy of immune plasma is closely correlated with the neutralizing (N) antibody titer to Lassa virus, expressed as a $\log_{10}$ neutralization index, or LNI. Unlike the N-antibody response to many viral infections, Lassa N-antibody evolves late in convalescence in both animal models and in human patients.

Other humoral antibody responses (such as the indirect fluorescent antibody test, or IFAT) to Lassa evolve rapidly, but do not reflect N-antibody titers, and are of little utility in selection of immune plasma for therapy.

Immune plasma with an LNI of 2 or more protected guinea pigs and monkeys when given soon after infection. Hyperimmune plasma with an LNI of more than 5 was protective and continued to be so even when diluted to a value of 2.

Human plasmas with LNI>2 likewise protected guinea pigs and monkeys. However, only a small proportion (18 of 130) of human plasmas selected on the basis of positive IFAT titers to Lassa had LNI>2, and none exceeded 3. Therefore, if it were possible to fractionate this low titer human plasma in a manner such that the IgG LNI was now greater than 2, this would make more feasible the possible clinical use of human IV IgG for treatment of Lassa fever.

2. Previous Work

Intravenous Human IgG:

We were the first to prepare animal and human IgG for intravenous administration exclusively by ion exchange chromatography (20,21,22). The methods developed have resulted in the preparation of equine anti-lymphoblast globulin, human IgG, and human hyperimmune CMV IgG. All these preparations are safe for IV administration in large doses and have been used in clinical trials for more than 10 years. There has been little or no evidence of systemic reactions (22,23), no evidence of hepatitis transmission and the use of equine ALG and the human hyperimmune CMV IgG in multicenter clinical trials have shown them both to be safe and efficacious (1,2,28,29). We previously prepared over 2000 grams of a human intravenous, pen-

tavalent, botulinal immune globulin for USAMRIID, Contract No. DAMD17-81-C-1120, "Preparation of a Homologous (Human) Intravenous, Botulinal Immune Globulin".

Human Botulinal Immune Globulin:

The purpose of fractionating hyperimmune, human, botulinal plasma was to isolate the specific botulinal toxin neutralizing antibodies and concentrate them in a state and purity such that: 1) their biological activity will be undiminshed (stable) on long-term storage (years), 2) they will be safe for intravenous administration and 3) they will possess the circulatory half-life and distribution of native undenatured plasma (immunoglobulins).

The ion exchange method of protein separation and purification is based on the relative charge of the protein and the resin and therefore on the isoelectric point of the proteins. However, immunoglobulins are heterogenous, complex, bifunctional proteins that differ from other plasma proteins in that they have broad rather than narrow isoelectric points--a reflection of their extreme molecular heterogeneity. In hyperimmunized individuals the predominant toxin neutralizing activity will be found in the molecules of the immunoglobulin IgG class--which also makes up 70-80% of the total humoral antibodies in plasma. Therefore, any method to isolate specific neutralizing activity must isolate IgG rather than IgA or IgM.

Further, IgG is composed of four subclasses--IgG_1, IgG_2, IgG_3, and IgG_4. Their relative concentration is of great practical importance since IgG_1 represents 60% of total IgG, IgG_2 30%, IgG_3 5% and IgG_4 2%. There is evidence that antibodies to certain groups or types of antigens can be limited to one or some of the IgG subclasses: antibodies to carbohydrates, dextran or levan have been found to be exclusively IgG_2 molecules, antibodies to antigens on cell membranes are predominately IgG_1 and IgG_3, and auto-antibodies to the blood clotting Factor VIII were identified as IgG_4. Antiplatelet antibodies in autoimmune thrombocytopenia purpura appear to be restricted to IgG_3. Recently, it has been reported that over 90% of the herpes virus neutralizing antibodies were also located in the IgG_3 subclass (24).

A number of observations have shown that in addition to their antibody properties, IgG molecules possess biologic activities that are controlled by their constant portion, the Fc fragment. These activities may be common to all four of the IgG subclasses or restricted to some. IgG is the only immunoglobulin class transmitted from the mother to the fetus. It seems that all four subclasses are transported across the placenta. Complement activation by the classic pathway is accomplished more readily by IgG_3 and IgG_1, than by IgG_2 whereas IgG_4 does not activate at all. These differences are not seen in the alternate pathway. Also, IgG_1 and IgG_3 are the only subclasses that can induce phagocytosis of opsonized antigenic particles. IgG_3 does not react with staphylococcal protein A. Finally, IgG subclasses differ in their metabolic behavior: IgG_3 is

more readily catabolized than the other three subclasses. Its biological half-life is 7 days compared to 21 days for IgG_1, IgG_2, and IgG_4.

While the IgG molecules have broad isoelectric ranges, each subclass does have a relatively restricted region. Thus, IgG_4 has the most acidic region, pI 5.7-6.2, IgG_1 the most basic, pI 6.8-9.5, IgG_2, pI 6.9-8.3, and IgG_3, pI 8.2-9.0.

Ideally, the most effective IV human, hyperimmune antibotulinal IgG should contain only the neutralizing antibody molecules. However, such a preparation could only be prepared by an affinity method involving immobilized botulinal toxins which are reacted with hyperimmune plasma and the specific antitoxin molecules eluted and concentrated. We have developed methods of isolating the predominant neutralizing activity which is reflected by a highly specific toxin neutralizing activity. To demonstrate this fact we have performed preparative isoelectric focusing of hyperimmune, human plasma eluting proteins from each segment and determined the toxin neutralizing activity to botulinal toxin A. This shows that the major neutralizing activity is located in the pI region from the most basic IgG subclass which also corresponds to the region of IgG_1 subclasses. Therefore, the ionic exchange method should isolate and concentrate this relatively high pI region protein. Comparison of plasma and IVBG-1A final product show that the highest toxin neutralizing activities of both hyperimmune plasma and final product were located in identical pI regions.

While IgG_3 is removed in our method by SiO_2 treatment, the toxin neutralizing activity is not appreciably affected. This loss of the subclass should have little effect since IgG_3 has the shortest half-life, 7 days vs. 21 days for IgG_1, and is lowest in relative concentrations, 5% vs. 60% of IgG_1.

Human Hyperimmune CMV IgG:

That the lack of IgG_3 is not a factor in CMV virus neutralizing activity is documented by our work in the prevention of cytomegalovirus (CMV) infection in bone marrow transplant recipients (1,2,7,28,29). A hyperimmune CMV globulin was prepared from high titer human plasma. This IgG was then administered in a controlled trial to bone marrow transplant recipients and the patients were monitored for CMV disease. There was no CMV infection in the treated group while 50% of the control group had documented CMV infection. There was also no CMV disease, i.e. interstitial pneumonia in the treated group, whereas 33% of the untreated group had interstitial pneumonia. We conclude that our preparation and this type of therapy are extremely effective in preventing CMV infection in the bone marrow transplant recipient and that other specific antiviral IgG's prepared by these methods should also be effective.

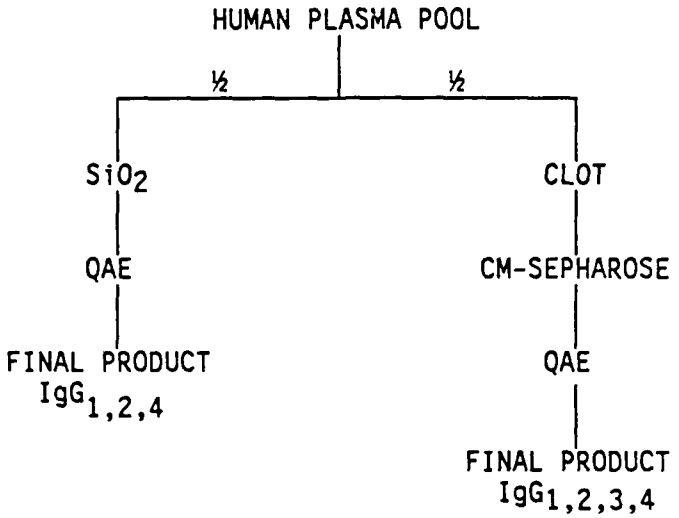
Human and Monkey Anti-Lassa IgG:

In collaboration with Dr. Peter Jahrling of USAMRIID and work completed under Contract No. DAMD17-84-C-4046, we isolated the IgG from marginally titered Lassa fever convalescent plasma as well as the IgG from high titer anti-Lassa monkey plasma. The purpose of this work was to determine whether our methods of fractionating IgG by ion exchange chromatography could isolate and concentrate the virus neutralizing activity of marginally titered human convalescent plasma, thereby converting ineffective, nonprotective material into a useful IgG for treatment. In addition, we wanted to determine whether these same procedures could be applied to high titer monkey plasma for the isolation of potent monkey IgG for possible use in treatment of Lassa fever. While the marginally titered human convalescent plasma was not protective, the IgG isolated from it was effective and resulted in complete protection of all animals tested when the log neutralizing index (LNI) was greater than 2. The monkey IgG also completely protected guinea pigs when the LNI was above 2. These preliminary findings are of importance since they demonstrate that 1) the LNI is a reliable assay of protective activity of both plasma and IgG and can be used as a measure of protective potency for these IgG preparations; 2) the IgG from marginally titered human plasma is protective, when the LNI is greater than 2; and 3) our fractionation methods can be used for isolating active and effective monkey IgG.

Part I

HUMAN ANTI-LASSA IgG POOL TWO (ALF-2)

On March 25, 1982 we received four units of frozen human Lassa fever convalescent plasma from USAMRIID. On May 4, 1983 we received another shipment of eighteen 100 cc sample vials of frozen human Lassa fever convalescent plasma. This material was combined which yielded a mixed plasma pool of 2.53 liters. This pool was divided into two equal portions for the production of two intact immune globulin final products as shown below.



Both of the final products were evaluated in animal and in vitro models at USAMRIID for the most effective viral neutralizing preparation.

Preparation of Intact IgG

The detailed processing of the plasma pool is presented in Figure 1, Fractionation Flow Sheet. One half of the initial pool was thawed at 4°C. This portion was used in the production of intact IgG.

The plasma pool was treated with sterile 40% calcium chloride at the ratio of one ml $CaCl_2$ per 100 ml of plasma. The plasma was allowed to clot in a 37°C incubator for four hours. Following clotting, the plasma-clot was centrifuged at 13,000 RPM (27,600 x g) for 45 minutes at 4°C. The supernatant was filtered through a sterile 28μ cloth.

The post clot plasma was dialyzed to pH 5.0 and concentrated. At this point, the plasma was divided into two equal portions to facilitate the fractionation procedure.

The first half of the pre CM-Sepharose plasma was centrifuged at 13,000 RPM (27,600 x g) for 20 minutes at 4°C. The supernatant was filtered through a sterile 28μ cloth. This plasma was applied to an equilibrated column of CM-Sepharose at pH 5.0. After eluting the first peak, a pH 8.2 buffer was used to elute the bound main protein peak.

The second half of the pre CM-Sepharose plasma was centrifuged, applied to a cleaned, equilibrated column of CM-Sepharose, and eluted as before.

The main IgG containing peaks of the two elutions were combined, concentrated, and dialyzed to pH 6.6.

The pre QAE protein was centrifuged at 13,000 RPM (27,600 x g) for 45 minutes at 20°C. The protein was filtered through a sterile 28μ cloth. This portion was applied to a QAE A-50 column (equilibrated) at pH 6.6. The post elution peak was pooled, concentrated and applied to another equilibrated column of QAE A-50, and eluted as before. Upon completion of elution, the entire protein peak was pooled.

The post QAE protein was concentrated and dialyzed into the final product. Before bottling, this protein was centrifuged at 13,000 RPM (27,600 x g) for 45 minutes at 20°C. The final product was filtered through a sterile 28μ cloth and then sterile filtered through a .22μ filter. The completed intact IgG, containing IgG subclasses 1,2,3,4 was sterile bottled in 10 cc glass vials at pH 6.8 and frozen at -20°C. Table I summarizes the yields on the final product. There were 53 vials at a protein concentration of 24.8 mg/ml with a total protein yield of 13.5 grams or 10.68 grams/liter plasma.

The final product was analyzed by high performance liquid chromatography (HPLC) using a TSK 3000SW gel filtration column with phosphate buffered saline (PBS) as a mobile phase (Figure 2). The HPLC showed a main peak to have a molecular weight of 166,000. Also visible were two small peaks at 372,000 and 269,000 molecular weights.

The final product was tested by radial immunodiffusion (RID). The proteins assayed were IgG, albumin, transferrin, alpha-2 macroglobulin, alpha-lipoprotein, and β-lipoprotein. The final product showed IgG but nothing with the other antisera.

The final product showed one band of IgG by immunoelectrophoreses (IEP) when reacted against anti-whole human serum (Figure 3).

Isoelectrofocusing (IEF) is a more sensitive way of determining the composition of the final product. The final product was run on an IEF gel, pH 3.5-9.5, and transferrin was visible at the concentration of 25 mg/ml. The IgG is typified by the broad band at the different pH regions corresponding to the heterogeneity of the gammaglobulins (Figure 4).

Enclosed is a chart showing the two final products and their respective yields. From the 1.3 liters of plasma a total of 13.5 grams $IgG_{1,2,3,4}$ was isolated for a yield of 17.4% (Table I).

Preparation of Intact IgG Lacking the IgG_3 Subclass

One half of the initial pool volume was thawed at 4°C. This portion was used in the production of intact IgG with the IgG_3 subclass reduced or removed.

The plasma pool was treated with synthetic silicon dioxide (SiO_2), with mixing, at the ratio of 15 grams SiO_2 per liter of plasma. The suspension was stirred with a motor driven rotor for one hour at room temperature. Following mixing, the plasma

was centrifuged at 13,000 RPM (27,600 x g) for 45 minutes at 4°C. The supernatant was filtered through a sterile 28μ cloth. The sedimented cake was resuspended in normal saline and centrifuged as before. This supernatant was filtered and combined with the first supernatant and the process repeated one more time.

The post SiO_2 absorbed pool was dialyzed to pH 6.6 and concentrated. The pre QAE plasma was centrifuged at 13,000 RPM (27,600 x g) for 45 minutes at 20°C. The plasma was filtered through a sterile 28μ cloth. This plasma was applied to an equilibrated column of QAE A-50 at pH 6.6. Upon completion of elution, the entire protein peak was pooled.

The post QAE pool was concentrated and dialyzed into final product. Before bottling, this protein was centrifuged at 13,000 RPM (27,600 x g) for 45 minutes at 20°C. The final product was filtered through a sterile 28μ cloth and then sterile filtered through a .22μ filter. The completed intact, $IgG_{1,2,4}$ was sterile bottled in 10 cc glass vials at pH 6.8 and frozen at -20° C. There were 46 vials at a protein concentration of 24.6 mg/ml with a total protein yield of 12.3 grams or 9.72 grams/liter plasma (Table I).

The final product was analyzed by HPLC using a TSK 3000 SW gel filtration column with PBS as a mobile phase. The HPLC graph showed a main peak to have a molecular weight of 166,000 (Figure 5).

The final product was tested by RID. The proteins assayed were IgG, albumin, transferrin, alpha-2 macroglobulin, alpha-lipoprotein, and β-lipoprotein. The final product showed a 61% yield of IgG with no reaction to the other antisera.

The final product showed a single band by IEP when reacted against anti-whole human serum (Figure 6).

The final product was run on an IEF gel, pH 3.5-9.5. Transferrin and alpha-2 macroglobulin were present at the concentration of 25 mg/ml (Figure 4).

Table I shows, for purpose of comparison, the two final products and their respective yields. From the 1.3 liters of plasma a total of 12.3 grams $IgG_{1,2,4}$ was isolated for a yield of 15.3% (Table I).

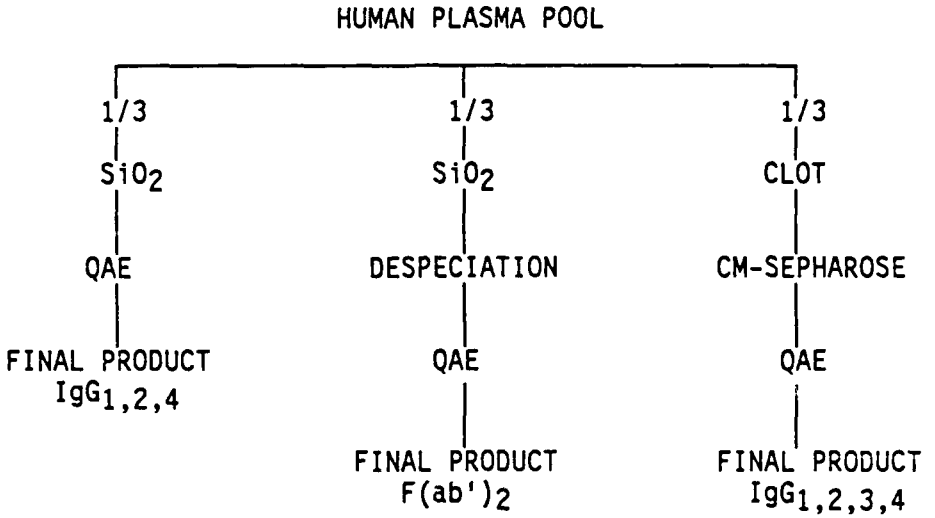
Protection of Monkeys and Guinea Pigs With Globulin Fractions

The protective efficacies of the ALF-2 unfractionated plasma, ALF-2 (CM) final product and ALF-2 (QAE) final product were determined in cynomolgus monkeys and guinea pigs infected with Liberian Lassa virus strains. The unfractionated convalescent plasma with an LNI of 1.6 failed to protect nonhuman primates from Lassa fever (Table II and Table III). The higher dose, 6 ml/kg, suppressed viremia, prolonged the mean time of death, but resulted in only one survivor of four treated monkeys and two survivors of five treated guinea pigs (Tables II and III).

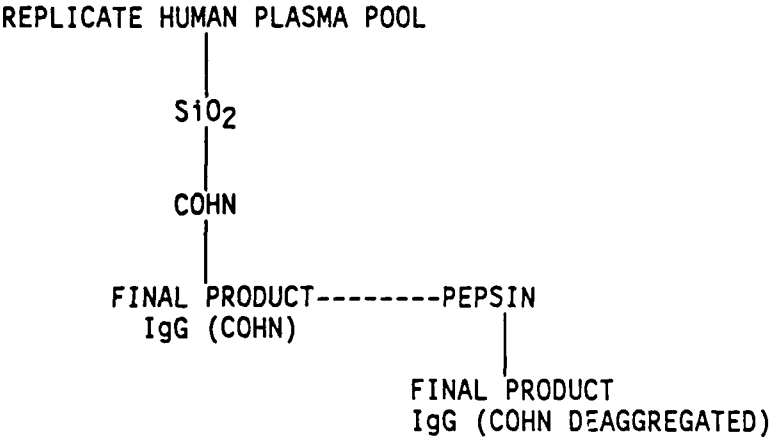
Both QAE and CM purified globulin final products, with LNI's of 3.0 and 3.5 respectively, protected monkeys and totally inhibited viremia when administered in high dose, 6 ml/kg (Table II). The QAE purified globulin protected guinea pigs when administered in high dose, 6 ml/kg, but was less effective at the lower dose of 3 ml/kg, where four of the treated guinea pigs survived (Table III). The CM purified globulin protected guinea pigs when administered in high dose, 6 ml/kg, and at a lower dose, 3 ml/kg (Table III).

HUMAN ANTI-LASSA IgG POOL THREE (ALF-3)

On April 24, 1984 we received 42 units of frozen human Lassa fever convalescent plasma from USAMRIID. The confirmation that all units were hepatitis B surface antigen negative was received the week of June 22, 1984. This material yielded a mixed plasma pool of 11.4 liters. This pool was divided into three equal portions for the production of two intact and one fragmented $F(ab')_2$ immune globulin final products as shown below.



On September 18, 1985, we received 16 units of frozen human Lassa fever convalescent plasma. This material yielded a replicated, mixed plasma pool of 3.8 liters. This pool was Cohn fractionated as shown below. One quarter of the final product IgG was treated with pepsin to remove aggregates.



All of these final products were evaluated in animal and in vitro models at USAMRIID for the determination of the most effective viral neutralizing preparation.

Preparation of Intact IgG

The detailed processing of the plasma pool is presented in Figure 7, Fractionation Flow Sheet. One third of the initial pool was thawed at 4°C. This portion was used in the production of intact IgG.

The plasma pool was treated with sterile 40% calcium chloride at the ratio of one ml $CaCl_2$ per 100 ml of plasma. The plasma was allowed to clot for eighteen hours, at 4°C, while stirring continuously with a motor driven rotor. Following clotting, the plasma and clots were centrifuged at 13,000 RPM (27,600 x g) for 45 minutes at 4°C. The supernatant was filtered through a sterile 28μ cloth.

The post clot plasma was dialyzed to pH 5.0 and concentrated. During the dialysis, the ultrafiltration membranes became plugged. The membranes were rinsed and cleaned, and diafiltration resumed. The membranes needed to be rinsed and cleaned for a total of 4 times. After the fourth cleaning, the diafiltration went smoothly.

The pre CM-Sepharose plasma was centrifuged at 13,000 RPM (27,600 X g) for 45 minutes at 20°C. The supernatant was filtered through a sterile 28μ cloth. This plasma was applied to an equilibrated column of CM-Sepharose at pH 5.0. After eluting the first peak, a pH 8.2 buffer was used to elute the main IgG protein peak. This main IgG containing peak was pooled, concentrated, and dialyzed to pH 6.6.

At this point, the protein was divided into two equal portions to facilitate the final fractionation procedure. The first half of the pre QAE protein was centrifuged at 13,000 RPM (27,600 X g) for 45 minutes at 20°C. The protein was filtered through a sterile 28μ cloth. This protein was applied to a QAE A-50 column (equilibrated) at pH 6.6. Upon completion of elution, the entire protein peak was pooled. The second half of the pre QAE protein was centrifuged, applied to another equilibrated column of QAE A-50, and eluted as before.

At this point the two post QAE pools were mixed together for dialysis and concentration into the final product. Before bottling, this protein was centrifuged at 13,000 RPM (27,600 X g) for 45 minutes at 20°C. The final product was filtered through a sterile 28μ cloth and then sterile filtered through a .22μ filter. The purified intact IgG, containing IgG subclasses 1,2,3,4, was sterile bottled in 20cc glass vials at pH 6.8 and frozen at -20°C. Table IV summarizes the yields on the final product. There were 70 vials at a protein concentration of 25.8 mg/ml with a total protein yield of 36.6 grams or 9.63 grams/liter plasma.

The final product was analyzed by high performance liquid chromatography (HPLC) using a TSK 3000SW gel filtration column with phosphate buffered saline (PBS) as a mobile phase (Figure 8). The HPLC showed a main peak to have a molecular weight of 161,000. Also visible were two small peaks at 335,000 and 260,000 molecular weights.

The final product was tested by radial immunodiffusion (RID). The proteins assayed were IgG, albumin, transferrin and alpha-2 macroglobulin. The final product showed IgG and a trace of transferrin, but nothing with the other antisera.

The final product showed a main band and a very faint band, indicating IgG and transferrin, respectively, by immunoelectrophoresis (IEP) when reacted against anti whole human serum (Figure 9).

Isoelectrofocusing (IEF) is a more sensitive way of determining the composition of the final product. The final product was run on an IEF gel, pH 3.5 - 9.5, and transferrin was present at the concentrations of 10 mg/ml and as is. In comparing the IEF's for the various final products, they all show the same broad banding in the different pH regions corresponding to the heterogeneity of the gamma globulins (Figure 10).

Enclosed is a chart showing the different final products and their respective yields. From the 3.8 liters of plasma a total of 36.6 grams $IgG_{1,2,3,4}$ was isolated for a yield of 15.3% (Table IV).

Preparation of Intact IgG Lacking the IgG_3 Subclass

One third of the initial pool volume was thawed at 4°C. This portion was used in the production of intact IgG with the IgG_3 subclass reduced or removed.

The plasma pool was treated with synthetic silicon dioxide (SiO_2), with mixing, at the ratio of 15 grams SiO_2 per liter of plasma. The suspension was stirred with a motor driven rotor for one hour at room temperature. Following mixing, the plasma was centrifuged at 13,000 RPM (27,600 X g) for 45 minutes at 4°C. The supernatant was filtered through a sterile 28μ cloth. The sedimented cake was resuspended in normal saline and centrifuged as before. This supernatant was filtered and combined with the first supernatant and the process was repeated one more time. The combined pools were mixed and a 20 ml sample was removed, sterile filtered, bottled at 27.6 mg/ml and frozen at -20°C to be sent to USAMRIID for post SiO_2 plasma (Table IV).

The post SiO_2 absorbed pool was dialyzed to pH 6.6 and concentrated. The plasma was then divided into two equal portions to facilitate the chromatography procedure. The first half of the pre QAE plasma was centrifuged at 13,000 RPM (27,600 X g) for 45 minutes at 20°C. The plasma was filtered through a sterile 28μ cloth. This plasma was applied to an equilibrated column of QAE A-50 at pH 6.6. Upon completion of elution, the entire protein peak was pooled. The second half of the pre QAE plasma was centrifuged and applied to another equilibrated column of QAE A-50, as before. The main protein peak was pooled.

At this point the two post QAE pools were mixed together for dialysis and concentration into one final product. Before bottling, this protein was centrifuged at 13,000 RPM (27,600 X g) for 45 minutes at 20°C. The final product was filtered through a sterile 28μ cloth and then sterile filtered through a .22μ filter. The purified intact, $IgG_{1,2,4}$, was sterile bottled in 20cc glass vials at pH 6.8 and frozen at -20°C. There were 69 vials at a protein concentration of 25.6 mg/ml with a total protein yield of 36.6 grams or 9.63 grams/liter plasma (Table IV).

The final product was analyzed by HPLC using a TSK 3000SW gel filtration column with PBS as a mobile phase. The HPLC graph showed a main peak to have a molecular weight of 157,000. Also visible was a small peak at 315,000 molecular weight (Figure 11).

The final product was tested by RID. The proteins assayed were IgG, albumin, transferrin, and alpha-2 macroglobulin. The final product showed a 70% yield of IgG with no reaction to the other antisera.

The final product showed a single band by IEP when reacted against anti-whole human serum (Figure 12).

The final product was run on an IEF gel, pH 3.5 - 9.5, and transferrin was present at the concentration of 10 mg/ml and as is (Figure 10).

Table IV shows, for purposes of comparison, the different final products and their respective yields. From the 3.8 liters of plasma a total of 36.6 grams of $IgG_{1,2,4}$ was isolated for a yield of 17.8% (Table IV).

Preparation of $F(ab')_2$

One third of the initial plasma pool volume was thawed and used for the production of $F(ab')_2$ immune globulin.

Dry silicon dioxide was slowly added, with mixing, to a final concentration of 15 grams SiO_2 per liter of plasma. This solution was mixed by a motor driven rotor for one hour at 4°C and then centrifuged at 13,000 RPM (27,600 X g) for one hour at 4°C. The supernatant was filtered through a sterile 28μ cloth. The precipitate was resuspended with normal saline and recentrifuged. This supernatant was filtered, combined with the first supernatant, and the process was repeated one more time.

At this point, the plasma was divided into two equal portions to facilitate the despeciation procedure. The first half of this pool was adjusted to pH 2.59 with .5N H_2SO_4 and pepsin was added at a concentration of 1% (g/g). The plasma temperature was raised to 45°C over a period of 15 minutes. At this point, the pH was adjusted to 3.90 with 1N NaOH and maintained for one hour by the addition of acid. The temperature was then lowered to 19°C and the plasma centrifuged at 13,000 RPM (27,600 X g) for one hour at 4°C. An amount of dry QAE A-50 equal to 2.5 times the amount of pepsin was added at room temperature and mixed for one hour. The QAE was removed by filtration through a 28μ sterile cloth. The solution was then diafiltered to pH 6.6 and concentrated. This protein was applied to an equilibrated column of QAE at pH 6.6. During the course of protein elution, the column had to be opened twice for removal of the top layer of QAE that was excessively saturated with protein. Upon completion of elution, the entire protein peak was pooled and sterile filtered.

The second half of the aerosil treated plasma was adjusted to a pH of 3.88 and pepsin was added at a 1% concentration. The plasma was heated to 45°C. The pH was maintained at 3.90 at this temperature for 60 minutes. The plasma was then cooled to 14°C and centrifuged at 13,000 RPM (27,600 X g) for one hour at 4°C. The pH was raised to 6.65 before the addition of dry QAE at a ratio of 5 grams QAE per gram of pepsin. This solution was mixed for one hour at room temperature and then filtered through a 28μ cloth. Following diafiltration and concentration, the protein was applied to a QAE column equilibrated to pH 6.6. The main protein peak was pooled and sterile filtered.

At this point the two post QAE pools were mixed together for concentration and dialysis into one final product. Before bottling, this protein was centrifuged

at 13,000 RPM (27,600 X g) for 45 minutes at 20°C. The final product was filtered through a sterile 28μ cloth and then sterile filtered through a .22μ filter. The completed $F(ab')_2$ was sterile bottled in 20 cc glass vials at pH 6.8 and frozen at -20°C. There were 40 vials at a protein concentration of 25.8 mg/ml with a total protein yield of 20.6 grams or 5.43 grams/liter plasma (Table IV).

The final product was analyzed by HPLC using a TSK 3000SW gel filtration column with PBS as a mobile phase and showed the main peak to have a molecular weight of 98,000. Also visible were small peaks at 28,000 and 16,000 molecular weights (Figure 13).

The final product showed a single band by immunoelectrophoresis when reacted against anti-whole human serum at concentrations of 25.8 mg/ml and 51.6 mg/ml (Figure 14).

The final product was also run on an IEF gel, pH 3.5 - 9.5 (Figure 10).

Table IV shows the different final products and their respective yields. From the 3.8 liters of plasma a total of 20.6 grams $F(ab')_2$ were produced for a yield of 9.6% (Table IV).

Preparation of Cohn Fractionated IgG

The detailed processing of the plasma pool is presented in Figure 7.

The plasma pool was treated with synthetic silicon dioxide, with mixing at the ratio of 15 grams SiO_2 per liter of plasma. The suspension was stirred with a motor driven rotor for one hour at room temperature. Following mixing, the plasma was centrifuged at 13,000 RPM (27,600 x g) for 45 minutes at 4°C. The supernatant was filtered through a sterile 28μ cloth and the sedimented cake was discarded.

The post SiO_2 adsorbed pool was split into 4 equal parts to accommodate the fractionation procedure. One quarter of the pool was adjusted to pH 6.8 with pH 4.0 sodium acetate buffer. The plasma was cooled in a salt/ice bath to +1°C without permitting ice to form. To the mixing plasma, cold (-10°C) 80% ethanol was added slowly to a final concentration of 25% ethanol. The plasma temperature was kept below 4°C during the addition. The plasma/ethanol mixture was allowed to mix for 30 minutes.

Precipitate I was removed by centrifugation at -5°C for 15 minutes at 13,000 RPM (27,600 x g). Supernatant I was discarded. Precipitate I was resuspended by the addition of shaved ice while gently swirling.

The remaining three-quarters of the post SiO_2 pool were treated with 80% ethanol, as before.

Upon completion of the Precipitate I resuspensions, all four pools were combined. The pool was adjusted to pH 5.1 with cold .05M acetic acid. Precipitate I was cooled to +1°C. Cold ethanol (80%) was added to the mixing pool to a final concentration of 10%.

Supernatant II was removed by centrifugation at 13,000 RPM (26,700 x g) for 15 minutes at -5°C. Precipitate II was discarded. The pH of Supernatant II was adjusted

to 7.4 with .5M sodium bicarbonate. The pool was divided into two equal portions to facilitate the fractionation procedure.

One half of Supernatant II was cooled to +1°C. Cold ethanol (80%) was added to a final concentration of 25%. The supernatant/ethanol mixture was allowed to mix for 30 minutes, keeping the temperature less than +4°C.

Precipitate III was removed by centrifugation at 13,000 RPM (26,700 x g) for 15 minutes at -5°C, and then resuspended by the addition of ice while gently swirling.

The second half of Supernatant II was treated with 80% ethanol, and centrifuged as before. Precipitate III was resuspended.

At this point, the resuspended precipitates were mixed together. Three-fourths of this pool was dialyzed and concentrated for the final product. Before bottling, the protein was centrifuged at 13,000 RPM (27,600 x g) for 45 minutes at 20°C. The final product was filtered through a sterile 28μ cloth and then sterile filtered through a .22μ filter. The completed Cohn fractionated IgG was sterile bottled in 20cc glass vials at pH 6.8 and frozen at -20°C. There were 41 vials at a protein concentration of 25.0 mg/ml with a total protein yield of 20.5 grams (Table IV).

The remaining quarter of the resuspended Precipitate III was treated with pepsin to reduce the aggregates. The pool was adjusted to pH 3.9 with .1M HCl. Pepsin was added at a concentration of 1:10,000 (g/g). The protein pool temperature was raised to 37°C, the activated pepsin was added and allowed to incubate for 15 minutes. At this point the pH was raised to 7.0 with 1.0 N NaOH.

The solution was dialyzed to pH 6.8 and concentrated for the final product. The completed deaggregated Cohn fractionated IgG was treated and bottled as before and frozen at -20°C. There were 13 vials at a protein concentration of 25.1 mg/ml for a total protein yield of 6.3 grams (Table V).

Both the final products were analyzed by HPLC using a TSK 3000SW gel filtration column with PBS as the mobile phase. The HPLC graph for the intact (Cohn) IgG showed the main peak to have a molecular weight of 157,000. Also visible was an aggregate peak at 742,000 and a low molecular weight peak of 5500 (Figure 15). The HPLC graph for the intact (deaggregated Cohn) IgG showed the main peak at 187,000 and an aggregate peak at 735,000 (Figure 16).

The final products were tested by RID. The proteins assayed were IgG, albumin, transferrin, IgA, IgM, and alpha-2 macroglobulin. The final products both showed a 44% yield of IgG with trace amounts of transferrin, albumin, IgA and IgM. There was no reaction to alpha-2 macroglobulin.

Each final product shows 3 precipitin bands by IEP when reacted against anti-whole human serum (Figure 7).

Table IV shows, for purposes of comparison, the different final products and their respective yields. From the 3.8 liters of plasma a total of 26.9 grams of IgG were isolated for a yield of 12.1% (Table IV).

Protection of Guinea Pigs With Globulin Fractions

The unfractionated convalescent plasma and the five different final products were compared for LNI and protective efficacy in guinea pigs infected with reference Sierra Leone Lassa (strain Josiah) and a Liberian strain (Z-132). Unfractionated plasma, with a LNI of 1.9 and 1.6, was marginally effective at 3 ml/kg, where 40% of the treated guinea pigs survived in each group for the two different challenge strains (Table V and Table VI). The protective efficacy of the unfractionated plasma was further reduced at the lower dose of 1.5 ml/kg, which resulted in one survivor of five treated guinea pigs when challenged with Josiah strain.

$F(ab')_2$ fragments had significantly reduced LNI, of 1.0 and 0.6 for the two challenge strains, relative to the plasma pool. This product was totally devoid of protective activity, administered at 1.5 ml/kg, and 3.0 ml/kg, when guinea pigs were challenged with both virus strains (Tables V and VI).

The intact CM purified globulin was marginally higher titered and more protective than the intact QAE purified globulin against both challenge virus strains (Table V and Table VI). All of the guinea pigs survived Z-132 challenge when administered intact CM globulin at 3 ml/kg, whereas 80% of the treated animals survived when administered intact QAE purified globulin. When the guinea pigs were treated with QAE purified globulin at a lower dose of 1.5 ml/kg, 60% of the animals survived the Z-132 challenge virus strain where 80% of the animals were protected with CM purified globulin (Table V). The QAE purified globulin protected 80% of the guinea pigs against the Josiah strain, when administered at 3 ml/kg, but only 60% of the animals survived when treated with CM purified globulin. The same protective efficacy was seen at the lower dose, 1.5 ml/kg, for CM purified globulin, but there was only one survivor of five QAE treated guinea pigs (Table VI).

Both the Cohn intact and deaggregated final products were highly protective, at 3 ml/kg, for the treated guinea pigs challenged against Z-132, Liberian virus strain. These final products were not tested at the lower dose (Tables V and VI).

The protective efficacies of the various preparations were enhanced by combination with suboptimal (and nontoxic) concentrations of ribavirin (Table VII). Ribavirin alone (20 mg/kg) was suboptimal where only one of five treated guinea pigs survived. The combination of $F(ab')_2$ with ribavirin suppressed viremia, prolonged the mean time of death, but did not enhance efficacy over ribavirin alone. Purified globulin by CM or QAE methods were shown to be protective when administered in doses of 3 ml/kg, but suboptimal at lower doses (Tables III, V and VI). The combination of ribavirin plus either CM or QAE purified globulins at 1.5 ml/kg significantly enhanced protection in the guinea pigs when challenged with Josiah strain virus. These results were reflected in the viremia patterns in the treated guinea pigs (Table VII).

MONKEY ANTI-LASSA IgG

We received 10 bottles of frozen high titer monkey anti-Lassa fever plasma which yielded a mixed plasma pool of 1.84 liters. This pool was divided into two equal portions for the production of one intact and one fragmented $F(ab')_2$ immune globulin final products. Each of these final products were evaluated in vitro and in animal models at USAMRIID for the determination of the most effective viral neutralizing preparation.

Preparation of Intact IgG

The detailed processing of the plasma pool is presented in Figure 18. One half of the pooled monkey plasma was thawed and used for the production of intact immune globulin.

The plasma pool was treated with sterile 40% calcium chloride at the ratio of one ml $CaCl_2$ per 100 ml of plasma. The plasma was allowed to clot for two hours in a 37°C water bath. Following the incubation, the clotted plasma was chopped up and centrifuged at 13,000 RPM (27,600 x g) for 45 minutes at 4°C. The supernatant was filtered through a sterile 28μ cloth.

The post clot plasma was dialyzed to pH 5.0 and concentrated. During the dialysis, the ultrafiltration membranes became plugged. The membranes were rinsed, cleaned, and diafiltration resumed. The membranes needed to be rinsed and cleaned for a total of three times. After the third cleaning, the diafiltration went smoothly.

At this point, the plasma was divided into two equal portions to facilitate the fractionation procedure. The first half of the pre CM-Sepharose plasma was centrifuged at 13,000 RPM (27,600 x g) for 45 minutes at 20°C. The supernatant was filtered through a sterile 28μ cloth. The plasma was applied to an equilibrated column of CM-Sepharose at pH 5.0. After the first peak was eluted, the buffer was switched to a pH 8.2 solution to elute the main peak. The main IgG protein peak was pooled. The second half of the pre CM-Sepharose plasma was centrifuged and applied to a cleaned and re-equilibrated column of CM-Sepharose at pH 5.0. The main protein peak was collected after switching to pH 8.2 buffer, as before.

At this point the two pH 8.2 main IgG containing peaks were mixed together for dialysis and concentration into pH 6.6 buffer. The pre QAE protein was centrifuged at 13,000 RPM (27,600 x g) for 45 minutes at 20°C. The protein was filtered through a sterile 28μ cloth. This protein was applied to a QAE A-50 column (equilibrated) at pH 6.6. Upon completion of elution, the entire protein peak was pooled for concentration and dialysis into the final product.

Before bottling, the final product was centrifuged at 13,000 RPM (27,600 X g) for 45 minutes at 20°C, filtered through a 28μ cloth, and then sterile filtered through a .22μ filter. The completed intact IgG was sterile bottled in 10cc glass vials at pH 6.8 and frozen at -20°C. Table VIII summarizes the yields on the final product. There were 28 vials at a protein concentration of 25.1 mg/ml with a total protein yield of 7.2 grams or 7.86 grams/liter plasma.

The final product was analyzed by HPLC using a TSK 3000SW gel filtration column with PBS as a mobile phase (Figure 19). The HPLC showed a main peak to have a molecular weight of 154,000. Also, visible were two small peaks at 323,000 and 22,000 molecular weights.

The final product showed one main band, indicating IgG by IEP when reacted against antibody to whole monkey serum (Figure 20).

Enclosed is a chart showing the different final products and their respective yields. From the 920 mls of plasma, a total of 7.23 grams IgG were isolated for a yield of 10.4% (Table VIII).

Preparation of $F(ab')_2$

One half of the pooled monkey plasma was thawed and used for the production of $F(ab')_2$ immune globulin.

Dry silicon dioxide was added to the pooled plasma to a final concentration of 15 grams SiO_2 per liter of plasma. This solution was mixed for one hour at 4°C and then centrifuged at 13,000 RPM (27,600 X g) for 40 minutes at 4°C. The supernatant was filtered through a 28μ nylon cloth. The precipitate was resuspended with normal saline and recentrifuged, as before. The supernatant from this solution was filtered and combined with the first supernatant. The precipitate was resuspended, centrifuged, and filtered one more time.

All three supernatants made up the predigestion pool. This pool was adjusted to pH 3.90 before the addition of purified pepsin to a final concentration of 1% (gram:gram). The temperature was raised to 45°C over a 15 minute period and maintained for 60 minutes, throughout which time the pH was kept at 3.90 with the addition of .5N H_2SO_4. The temperature was lowered to 18°C. The digested plasma was centrifuged at 13,000 RPM (27,600 X g) for 60 minutes at 4°C and then filtered through a sterile 28μ cloth. The post spin plasma was adjusted to pH 6.6 with the addition of saturated TRIS base. An amount of dry QAE A-50 equal to 2.5 times the amount of pepsin was added to the supernatant and allowed to mix for one hour at room temperature. The QAE was removed by filtration through a 28μ nylon cloth. The solution was then concentrated and diafiltered to pH 6.6 for application to an equilibrated column of QAE A-50. The main protein peak eluted from the column was pooled. The tailings from the column were also collected and pooled separately.

The main protein peak was concentrated and dialyzed into the final product. Before bottling, this protein was centrifuged at 13,000 RPM (27,600 X g) for 45 minutes at 20°C. The final product was filtered through a sterile 28μ cloth and then sterile filtered through a .22μ filter. The purified $F(ab')_2$ immune globulin was sterile bottled in 10cc glass vials at pH 6.8 and frozen at -20°C. Table VIII summarizes the yields on the final products. There were 18 vials at a protein concentration of 25.7 mg/ml with a total protein yield of 4.65 grams or 5.06 grams/liter plasma.

The tailings from the QAE run were concentrated, but not dialyzed. Before bottling, this protein was centrifuged at 13,000 RPM (27,600 X g) for 45 minutes at 20°C. The tailings were filtered through a sterile 28μ cloth and then sterile filtered through a .22μ filter. The $F(ab')_2$ tailings were sterile bottled in 10cc glass vials at pH 6.6 at a concentration of 4.7 mg/ml and frozen at -20°C (Table VIII).

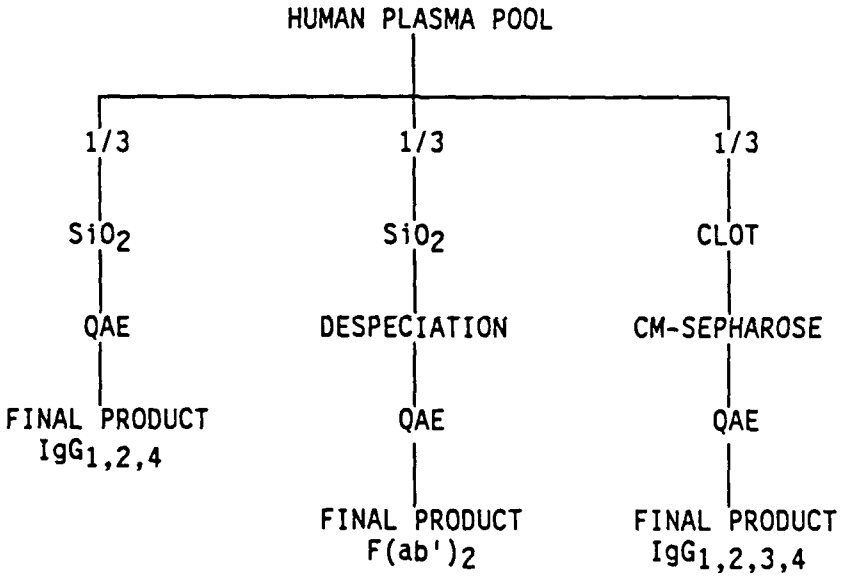
The $F(ab')_2$ final product was analyzed by HPLC, using a TSK 3000SW gel filtration column with PBS as a mobile phase, which showed one main peak with a molecular weight of approximately 96,000 and small peaks at 234,000, 43,000, and 26,000 molecular weights (Figure 21). From this analysis, the $F(ab')_2$ appears to be completely free of intact IgG.

By the method of immunoelectrophoresis, it can be seen that there is a small contaminating band in the β1, mobility region when the $F(ab')_2$ is reacted with anti-whole monkey serum (Figure 22). When anti-monkey IgG is used as antisera, only one precipitin line appears (Figure 23). The protective efficacy results for the monkey preparations are pending from USAMRIID.

Part II

HUMAN ANTI-HEMORRHAGIC IgG (AHF-1)

On February 7, 1985 we received 30 units of frozen human Argentinian Hemorrhagic fever convalescent plasma from USAMRIID. Some of these units and loop samples were returned to USAMRIID for HTLV-III testing. The confirmation that the remaining units were HTLV-III antibody negative was received June 19, 1985. On June 25, 1985 we received 14 more units of Argentinian Hemorrhagic fever convalescent plasma that were HTLV-III antibody negative. All units were hepatitis B surface antigen negative. From the 36 units that were retained, a 10 ml sample was removed from each unit, and the remaining material yielded a mixed plasma pool of 7.07 liters. A 200 ml samples from the mixed plasma pool was removed and sent with the individual unit sample to USAMRIID for testing. The remaining pool of 6.87 liters was divided into three equal portions for the production of two intact and one fragmented $F(ab')_2$ immune globulin final products as shown below.



On June 4, 1986 we received 20 units more of Argentinian Hemorrhagic fever plasma, that were negative for HTLV-III antibody, and hepatitis B surface ar[illegible] This material yielded a replicated, mixed plasma pool of 4.68 liters. Two t[illegible]rcent of the mixed plasma pool was removed, and the remaining 3.744 liters were despeciated, as before, to produce a fragmented $F(ab')_2$ immune globulin.

All of these final products were evaluated in animal and in vitro models at USAMRIID for the determination of the most effective viral neutralizing preparation.

Preparation of Intact IgG

The detailed processing of the plasma pool is presented in Figure 24, Fractionation Flow Sheet. One third of the initial pool was thawed at 37°C. This portion was used in the production of intact IgG.

The plasma pool was treated with sterile 40% calcium chloride at the ratio of one ml $CaCl_2$ per 100 ml of plasma. The plasma was allowed to clot for two hours at 37°C.

Following clotting, the plasma-clot material was centrifuged at 13,000 RPM (27,600 x g) for 45 minutes at 4°C. The supernatant was filtered through a sterile 28μ cloth.

The post clot plasma was dialyzed to pH 5.0 and concentrated. At this point, the plasma was divided into two equal portions to facilitate the fractionation procedure.

The pre CM-Sepharose plasma was centrifuged at 13,000 RPM (27,600 x g) for 45 minutes at 20°C. The supernatant was filtered through a sterile 28μ cloth. This plasma was applied to an equilibrated column of CM-Sepharose at pH 5.0. After eluting the first peak, a pH 8.2 buffer was used to elute the main IgG protein peak.

The second half of the pre CM-Sepharose plasma was centrifuged, applied to a cleaned, equilibrated column of CM-Sepharose, and eluted as before.

The main IgG containing peaks of the two elutions were combined, concentrated, and dialyzed to pH 6.6. The pre QAE protein was centrifuged at 13,000 RPM (27,600 x g) for 45 minutes at 20°C. The protein was filtered through a sterile 28μ cloth. This protein was applied to a QAE A-50 column (equilibrated) at pH 6.6. Upon completion of elution, the entire protein peak was pooled.

The post QAE protein was dialyzed and concentrated into the final product. Before bottling, this protein was centrifuged at 13,000 RPM (27,600 x g) for 45 minutes at 20°C. The final product was filtered through a sterile 28μ cloth and then sterile filtered through a .22μ filter. The purified intact IgG, containing IgG subclasses 1,2,3,4 was sterile bottled in 20 cc glass vials at pH 6.8 and frozen at -20°C. Table IX summarizes the yields on the final product. There were 25 vials at a protein concentration of 25.4 mg/ml with a total protein yield of 12.2 grams or 5.32 grams/liter plasma.

The final product was analyzed by high performance liquid chromatography (HPLC) using a TSK 3000SW gel filtration column with Phosphate Buffered Saline (PBS) as a mobile phase (Figure 25). The HPLC showed a main peak to have a molecular weight of 158,000. Also visible were two small peaks at 353,000 and 254,000 molecular weights.

The final product was tested by radial immunodiffusion (RID). The proteins assayed were IgG, albumin, transferrin and alpha-2 macroglobulin. The final product showed a 71% IgG yield, and nothing with the other antisera.

The final product showed a single band by immunoelectrophoresis (IEP) when reacted against whole human serum.

Immunoelectrofocusing (IEF) is a more sensitive way of determining the composition of the final product. The final product was run on an IEF gel, pH 3.5-9.5, and transferrin was present at the concentration of 25.4 mg/ml. In comparing the IEF's for the various final products, they all show the same broad banding in the different pH regions (Figure 27).

Enclosed is a chart showing the different final products and their respective yields (Table IX). From the 2.3 liters of plasma a total of 12.2 grams $IgG_{1,2,3,4}$ was isolated for a yield of 10% (Table IX).

Preparation of Human Argentinian Hemorrhagic Fever Intact IgG Lacking the IgG_3 Subclass

One third of the initial pool volume was thawed at 4°C. This portion was used in the production of intact IgG with the IgG_3 subclass reduced or removed.

The plasma pool was treated with synthetic silicon dioxide (SiO_2), with mixing, at the ratio of 15 grams SiO_2 per liter of plasma. The suspension was stirred with a motor driven rotor for one hour at room temperature. Following mixing, the plasma was centrifuged at 13,000 RPM (27,600 x g) for 45 minutes at 4°C. The supernatant was filtered through a sterile 28μ cloth. The sedimented cake was resuspended in normal saline and centrifuged as before. This supernatant was filtered and combined with the first supernatant and the process was repeated one more time.

The post SiO_2 absorbed pools were combined and dialyzed to pH 6.6 and concentrated. The pre QAE plasma was centrifuged at 13,000 RPM (27,600 x g) for 45 minutes at 20°C. The plasma was filtered through a sterile 28μ cloth. This plasma was applied to an equilibrated column of QAE A-50 at pH 6.6. Upon completion of elution the entire protein peak was pooled.

The post QAE protein was dialyzed and concentrated into final product. Before bottling, this protein was centrifuged at 13,000 RPM (27,600 x g) for 45 minutes at 20°C. The final product was filtered through a sterile 28μ cloth and then sterile filtered through a .22μ filter. The purified intact, $IgG_{1,2,4}$ was sterile bottled in 20 cc glass vials at pH 6.8 and frozen at -20°C. There were 22 vials at a protein concentration of 25.5 mg/ml with a total protein yield of 10.8 grams or 4.73 g/l plasma (Table IX).

The final product was analyzed by HPLC using a TSK 3000SW gel filtration column with PBS as the mobile phase. The HPLC graph showed a main peak to have a molecular weight of 158,000. Also visible was a small peak at 320,000 molecular weight (Figure 28).

The final product was tested by RID. The proteins assayed were IgG, albumin, transferrin, and alpha-2 macroglobulin. The final product showed a 60% yield of IgG with no reaction to the other antisera.

The final product showed a single band by IEP when treated against whole human serum (Figure 29).

The final product was run on an IEF gel, pH 3.5 and 9.5. Transferrin and alpha-2 macroglobulin were present at 25.5 mg/ml but not at 10 mg/ml. In comparing the IEF's for the various final products, they all show the same broad banding in the different pH regions (Figure 27).

Table IX shows, for purposes of comparison, the different final products and their respective yields. From the 2.3 liters plasma a total of 10.8 grams of $IgG_{1,2,4}$ was isolated for a yield of 8.6% (Table IX).

Preparation of Human Argentinian Hemorrhagic Fever $F(ab')_2$

One third of the initial plasma pool volume was thawed and used for the production of $F(ab')_2$ immune globulin.

Dry silicon dioxide was slowly added, with mixing, to a final concentration of 15 grams SiO_2 per liter of plasma. This solution was mixed by a motor driven rotor for one hour at 4°C. Following mixing, the suspension was centrifuged at 13,000 RPM (27,600 x g) for one hour at 4°C. The supernatant was filtered through a sterile 28μ cloth. This supernatant was filtered and combined with the first supernatant and the process was repeated one more time.

All three supernatants made up the predigestion pool. The post SiO_2 plasma was adjusted to pH 3.9 with .5N HCl and pepsin was added at a concentration of 1% (g/g). The plasma temperature was raised to 45°C over a period of 15 minutes. The pH was maintained at 3.90 for one hour by the addition of acid. The temperature was then lowered to 15°C and the plasma centrifuged at 5000 RPM (7280 x g) for 45 minutes at 10°C. The supernatant was filtered through a sterile 28μ cloth. This filtered plasma was adjusted to pH 6.6 with saturated TRIS base. An amount of dry QAE A-50 equal to five times the amount of pepsin was added at room temperature and mixed for one hour. The QAE was removed by filtration through a 28μ sterile cloth. The solution was diafiltered to pH 6.6 and concentrated. This protein was applied to an equilibrated column of QAE at pH 6.6. Upon completion of elution, the entire protein peak was pooled.

The post QAE protein was dialyzed and concentrated into final product. Before bottling, this protein was centrifuged at 13,000 RPM (27,600 x g) for 45 minutes at 10°C. The final product was filtered through a sterile 28μ cloth and then sterile filtered through a .22μ filter. The purified $F(ab')_2$ was sterile bottled in 20 cc glass vials at pH 6.8 and frozen at -20°C. There were 14 vials at a protein 25.5 mg/ml with a total protein yield of 7.3 grams or 3.21 grams/liter plasma (Table IX).

The final product was analyzed by HPLC using a TSK 3000SW gel filtration column with PBS as a mobile phase and showed the main peak to have a molecular weight of 96,000 (Figure 30).

By IEP, the final product showed a single band when reacted against whole human serum (Figure 31).

The final product was run on an IEF gel, pH 3.5-9.5. In comparing the IEF's for the various final products, they all show the same banding in the different pH rgions (Figure 27).

Table IX shows the different final products and their respective yields. From the 2.3 liters of plasma a total of 7.3 grams $F(ab')_2$ was produced for a yield of 6.1% (Table IX).

Protection of Guinea Pigs With Globulin Fractions

The unfractionated plasma and three different final products were compared for protective efficacy in guinea pigs infected with Junin virus. Unfractionated plasma was marginally effective in protecting guinea pigs against Junin virus (Table X).

Half of the guinea pigs survived and 3% of the animals exhibited paralysis when the plasma was administered at low and high doses, 6000 Therapeutic units (T.U.) and 30,000 T.U. respectively.

$F(ab')_2$ fragments were totally devoid of protective activity for both low and high doses (Table X).

The intact CM purified globulin and intact QAE purified globulin were totally protective at the low dose, 6000 T.U., when guinea pigs were challenged with Junin virus. None of the animals exhibited any paralysis (Table X).

Part III

HIV SPIKING EXPERIMENTS

On September 11, 1986 a collaborative project was started involving USAMRIID, MALG and the California Department of Health, Berkeley, CA. The human plasma was mixed types of HIV negative and Hepatitis B surface antigen negative plasma purchased from the St. Paul Red Cross. This was used in eight of the experiments. Two experiments used HTLV-III antibody positive human plasma which was supplied by the California Department of Health. All experiments and testing were performed at the Berkeley location.

Assay of Aliquots for Infectivity Rather than Antigenicity

The purpose of the study was to determine the elimination of HIV infectivity by the plasma fractionation process(es). Such elimination may result either from (1) physical removal of the infectious virions, or from (2) "chemical" inactivation of infectivity without physical removal of the virions. An infectivity assay by itself cannot distinguish between these possibilities. Quantitation of residual viral antigens, along with the infectivity data, could theoretically provide relevant information. This approach was found not to be meaningful, however, for the following reasons:

(1) In order to provide a wide safety margin, the plasma fractionation process should be capable of removing many logs of viral infectivity. The infectivity assay has an arbitrarily great "dynamic range" (dependent only upon the starting titer of the virus) over which such a decrease may be documented.

(2) Infectivity determinations can detect the survival of a single TCID-50 of virus. Antigen detection, even with the most sensitive antigen-capture ELISA techniques available, is far less sensitive. For example, less than 10% of patient specimens positive by infectivity assay (in PBLs) are positive by antigen-capture assay. Thus, antigen detection is a less informative measure of elimination of HIV by the plasma fractionation.

(3) The virus stock contains, in addition to infectious virus, a mixture of intact virions and soluble viral antigen molecules. Antigen-capture ELISA assays cannot distinguish between these, and a drop in viral antigen cannot therefore be equated with a loss of intact virions.

TCID-50 Assay Using MT-2 Cells

The MT-2 TCID-50 assay is based upon microscopic visual scoring of viral cytopathic effects ("cpe"). It was chosen for being the most sensitive and quantitative assay available. Alternative assays include: (1) those using other host cells, such as H-9 cells or primary human peripheral blood lymphocytes (PBLs); and (2) use of other end-points, such as immunofluorescence, reverse transcriptase or viral antigen expression.

Although PBLs are most sensitive for growth of HIV directly from clinical specimens, the HTLV-IIIB strain of HIV used in these experiments is thoroughly adapted to expression in continuous cell lines such as MT-2. Observation of cpe was cho-

sen as the most sensitive end-point to indicate HIV infectivity in the MT-2 cell system. For example, by observation of cpe, higher titers are seen than when immunofluorescence is used as an endpoint. This is true partly because the entire cell sheet is examined in the former case, while only a portion of the cells can be included in the aliquot examined by immunofluorescence. We have also shown in numerous experiments that cpe precedes the expression of HIV antigen detectable in MT-2 cultures by antigen-capture ELISA.

In order to make the MT-2 assay quantitative, quadruplicate culture wells are inoculated with each serial log dilution of sample. The presence of any cpe in a culture well is scored as positive for purposes of a Reed-Muench calculation to obtain end-point titers expressed in tenths of a log.

Experiment #1

350 ml of human plasma was concentrated and dialyzed to a volume of 185 ml then spiked with 19 ml of $10^{9.2}$ TCID-50/ml stock virus (Virus Lot #7-10) immediately prior to the column step.

Methods:

Human plasma was treated with wet SiO_2 at a concentration of 21 grams/liter. This was mixed and then centrifuged to remove the SiO_2-bound protein pellet.

The post SiO_2 plasma was concentrated and dialyzed on an ultrafiltration membrane of 10,000 molecular weight into pH 6.6 imidazole-acetate buffer.

A column was packed with QAE A-50 Sephadex and equilibrated with pH 6.6 imidazole-acetate buffer.

The pre QAE plasma was spiked with 18.5 ml of 1 x $10^{9.2}$ TCID-50/ml stock virus. The unbound protein peak, IgG, was eluted with pH 6.6 imidazole-acetate buffer.

After the main protein peak was eluted, switched to pH 4.0 sodium-acetate buffer to remove bound proteins. A pH 2.0 sodium-chloride buffer followed to finish cleaning the gel.

Results:

There was no virus recovered in the IgG containing peak or in the preceeding void volume (Figure 28). After the unbound peak was eluted, switched to the pH 4.0 sodium-acetate buffer to remove the bound proteins. There was no virus recovered in the void volume. When the bound protein began to elute off the column, a small amount of virus, 1.4 logs, was recovered in the first pH 4.0 elution pool (Figure 28). In the remaining pH 4.0 elution pools and the pH 2.0 elution, there was no virus recovered. See Flow Sheet Experiment #1.

Experiment #2

Human plasma spiked with HTLV-III at the start of processing.

Methods:

350 ml of human plasma was spiked with 35 ml of 1 x $10^{7.7}$ TCID-50/ml stock virus (Virus Lot #7-10). This post spike plasma was treated with 21 grams/liter wet SiO_2. The slurry was mixed and centrifuged to remove the SiO_2-bound protein pellet.

The post SiO_2 plasma was concentrated and dialyzed into pH 6.6 imidazole-acetate buffer on an ultrafiltration membrane of 10,000 molecular weight.

A column was packed with QAE A-50 sephadex and equilibrated with pH 6.6 imidazole-acetate.

The pre QAE plasma was applied to the column. The unbound peak, IgG, was eluted with pH 6.6 imidazole-acetate buffer.

After the main protein peak was eluted, changed to pH 4.0 sodium-acetate buffer to remove bound proteins. A pH 2.0 sodium-chloride buffer followed to finish cleaning the gel.

Results:

Approximately 1 log of virus was removed at each of the fractionation steps up to the QAE. There was no virus recovered in the initial void volume or in the main IgG containing peak (Figure 28). After the unbound peak eluted, switched to the pH 4.0 sodium-acetate buffer to remove the bound proteins. There was no virus recovered in the void volume. When the bound protein began to elute off the column, a small amount of virus, 1.2 logs, was recovered in the first pH 4.0 elution pool (Figure 28). In the remaining pH 4.0 elution pools and the pH 2.0 elution, there was no virus recovered. See Flow Sheet Experiment #2.

Experiment #3

HIV positive plasma, with a fluorescent antibody titer of 1:100,000, spiked with HTLV-III at the start of processing.

Methods:

150 ml of HIV positive plasma was spiked with 15 ml of 1 X $10^{8.7}$ TCID-50/ml stock virus (Virus Lot #7-10). This post spike plasma was treated with wet SiO_2 at a concentration of 21 grams/liter plasma. The slurry was mixed and centrifuged to remove the SiO_2-bound protein pellet.

The post SiO_2 plasma was concentrated and dialyzed into pH 6.6 imidazole-acetate on an ultrafiltration membrane of 10,000 molecular weight.

A column was packed with QAE A-50 sephadex and equilibrated with pH 6.6 imidazole-acetate buffer.

The pre QAE plasma was applied to the column. The unbound IgG peak was eluted with pH 6.6 imidazole-acetate buffer.

After the main protein peak was eluted, changed to pH 4.0 sodium acetate buffer

to remove bound proteins. A pH 2.0 sodium chloride buffer followed to finish cleaning the gel.

Results:

There was an initial decrease of 4.7 logs of virus when it was added to the high titer plasma, due to the formation of immune complexes.

There was another 1.5 log decrease of virus through the SiO_2 removal. The virus concentration of the pre QAE plasma was 1.5 TCID-50/ml. There was no virus recovered in the main IgG containing peak. There was also no virus recovered in any of the pH 4.0 elution fractions or the pH 2.0 elution. See Flow Sheet Experiment #3.

Experiment #4

HIV positive plasma, with a fluorescent antibody titer of 1:10,000, spiked with HTLV-III at the start of processing and prior to the column.

Methods:

150 ml of HIV positive plasma was spiked with 15 ml of 1 X $10^{9.0}$ TCID-50/ml stock virus (Virus Lot #6-7). This post spike plasma was treated with wet SiO_2 at a concentration of 21 grams/liter plasma. This slurry was mixed and centrifuged to remove the SiO_2-bound protein pellet.

The post SiO_2 plasma was concentrated and dialyzed on an ultrafiltration membrane of 10,000 molecular weight, into pH 6.6 imidazole-acetate buffer.

A column was packed with QAE A-50 sephadex and equilibrated with pH 6.6 imidazole-acetate buffer.

90 ml of the pre QAE plasma was again spiked with 9 ml of 1 X $10^{8.2}$ TCID-50/ml stock virus (Virus Lot #6-7). This plasma was applied to the column. The unbound IgG peak was eluted with pH 6.6 imidazole-acetate buffer.

After the main protein peak was eluted, a pH 4.0 sodium-acetate buffer was used to remove bound proteins. A pH 2.0 sodium-chloride buffer followed to finish cleaning the gel.

Results:

There was approximately a 4 log decrease of virus during the processing leading to the QAE A-50 column when the plasma was spiked again. There was no virus recovered in the main IgG containing peak or in the preceeding void volume (Figure 28). After the unbound peak was done eluting, switched to the pH 4.0 sodium-acetate buffer to remove the bound proteins. There was no virus recovered in the void volume. When the bound protein began to elute off the column, a small amount of virus, 0.7 logs, was recovered in the first pH 4.0 elution pool (Figure 28). In the remaining pH 4.0 elution peaks and the pH 2.0 elution, there was no virus recovered. See Flow Sheet Experiment #4.

Experiment #5

Human plasma spiked with HTLV-III prior to wet SiO_2 addition.

Methods:

180 ml of human plasma was spiked with 18 ml of 1 X $10^{7.7}$ TCID-50/ml stock virus (Virus Lot #6-7). The plasma was treated with wet SiO_2 at 21 grams/liter plasma. The slurry was mixed and centrifuged to remove the SiO_2-bound protein pellet.

Results:

There was a reduction of 1.5 logs of virus from spiking to post centrifugation. See Flow Sheet Experiment #5.

Experiment #6

To determine the viral removing capacity of wet QAE by batching.

Methods:

180 ml of human plasma, which had previously been SiO_2 treated, was seeded with 18 ml of stock virus at 1 X $10^{8.5}$ TCID-50/ml (Virus Lot #6-7) to give a starting titer of 1 X $10^{7.5}$. QAE A-50 which was previously swollen in normal saline was added to the plasma at a ratio of .08 grams QAE per gram protein. The mixture was stirred for one hour and then filtered to remove the QAE.

Results:

The wet QAE batch addition to the plasma accounted for a 0.8 log reduction in titer. See Flow Sheet Experiment #6.

Experiment #7

To determine the viral removing capacity of dry QAE by batching.

Methods:

85 ml of human plasma, which had previously been SiO_2 treated, was seeded with 9 ml of stock virus at 1 X $10^{8.5}$ TCID-50/ml (Virus Lot #6-7) to give a starting titer of 1 X $10^{7.4}$. Dry QAE A-50 was added to the plasma at a ratio of .08 grams QAE per gram protein. The mixture was stirred for one hour and then filtered to remove the QAE.

Results:

The dry QAE batch reduced the titer of the plasma by 0.2 logs. See Flow Sheet Experiment #7.

Experiment #8

To determine the effect of heating virus spiked plasma at 60°C.

Methods:

100 ml of human plasma, which had previously been SiO_2 treated, was seeded with 10 ml of stock virus at 1 X $10^{8.5}$ TCID-50/ml (Virus Lot #6-7) to give a starting titer of 1 X $10^{7.9}$. The plasma was immediately placed in a water bath at 60°C. Aliquots were removed for testing at 5, 10, 20, 40, and 60 minutes.

Results:

Heating the plasma for 5 minutes caused a 0.5 log reduction in titer. After 10 minutes the original titer had been reduced by 3.2 logs. After 20 minutes the initial titer had been reduced by 5.5 logs. No live virus was recovered in either the 40 minute or 60 minute sample. See Flow Sheet Experiment #8.

Experiment #9

To determine the effect of heating pepsin-treated, virus-spiked plasma at 60°C.

Methods:

100 ml of human plasma which had previously been SiO_2 treated was adjusted to pH 4.3 and spiked with 10 ml of stock virus at 1 X $10^{8.4}$ TCID-50/ml (Virus Lot #6-7) to give a starting titer of 1 X $10^{7.7}$. Pepsin was then added at a ratio of 1:10,000 (mg pepsin:mg total protein). The plasma mixture was immediately placed in a 60°C water bath. Aliquots were removed for testing at intervals of 5, 10, 20, 40 and 60 minutes. Each sample was adjusted to neutral pH by the addition of 1N NaOH.

Results:

After 5 minutes the virus titer had been reduced by 1.5 logs. In the 10 minute sample the titer had been reduced by a total of 5.0 logs from the starting titer. Another log of virus titer was removed after 20 minutes, making the total 6.0 logs. After 40 minutes the titer was reduced by 6.5 logs. In the 60 minute sample, however, the reduction in titer was only 5.5 logs. See Flow Sheet Experiment #9.

Experiment #10

To determine the effect of heating pepsin-treated, virus-spiked, purified IgG.

Methods:

100 ml of purified human gamma globulin final product was seeded with 10 ml of stock virus at 1 X $10^{8.2}$ TCID-50/ml (Virus Lot #6-7) to give a starting titer of 1 X $10^{7.0}$. Pepsin was then added at a ratio of 1:10,000 (mg pepsin:mg IgG). This mixture was immediately placed in a 37°C water bath and aliquots were removed for testing at 2, 4, 6 and 18 hours.

Results:

After 2 hours, the virus titer was reduced by 3.0 logs. In the 4 hour sample, the total reduction in titer was 6.3 logs. The 6 hour sample, however, only showed a reduction of 5.0 logs. After 18 hours at 37°C with pepsin, no virus was recovered. See Flow Sheet Experiment #10.

Conclusion

Conventional and experimental methods for viral removal were investigated along with the step-wise operating procedures for MALG production to determine the best method for producing a safe, virus-free final product.

Heat inactivation of virus seeded plasma was accomplished, with total reduction in titer after 40 minutes at 60°C.

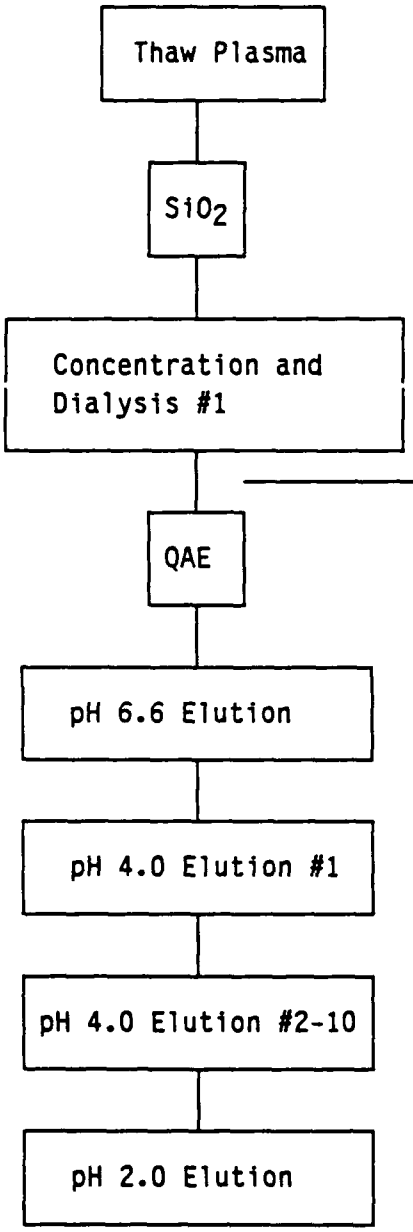
The combination of heat treatment and pepsin addition has been proposed as a method for viral inactivation. However, it was found that the results of the experiments were inconclusive and raised more questions than were answered. The virus spiked plasma which contained pepsin, retained 2.2 logs of virus titer after heating at 60°C, whereas the non-pepsin control was totally inactivated after only 40 minutes.

In examining separate processes in the MALG production, it was determined approximately how each could contribute to reduction in viral titers. Wet aerosil treatment at 21 grams/liter removes about two logs of virus titer. The addition of swollen QAE in a batch method also removed approximately one log of virus titer. The dialysis and concentration procedure using the 10,000 MW ultrafiltration membranes caused a reduction in virus titer of about one log, which is most likely due to non-specific binding to the polysulfone membrane.

The QAE column chromatography procedure in every experiment was capable of removing all of the "viral infectivity" that was applied. The highest load was in Experiment #1, in which the plasma was spiked immediately before the column. Based on that experiment, the QAE column was capable of binding $1.1 \times 10^{8.7}$ TCID-50 (8.74 logs) per gram of equilibrated QAE. The maximum binding capacity of QAE for HTLV-III was not determined. In each experiment the virus was released from the column with the onset of the pH 4.0 cleaning buffer, but then became inactivated at the low pH and could not be further detected.

FLOW SHEET EXPERIMENT #1

Negative plasma seeded with HTLV-III immediately prior to column



	TCID-50/ml[1]	Fraction Surviving[2]
HTLV-III spike	7.7	10^0
pH 6.6 Elution	NVR[3]	$<10^{-7.7}$
pH 4.0 Elution #1	1.4	$10^{-6.3}$
pH 4.0 Elution #2-10	NVR	$<10^{-6.7}$
pH 2.0 Elution	NVR	$<10^{-6.7}$

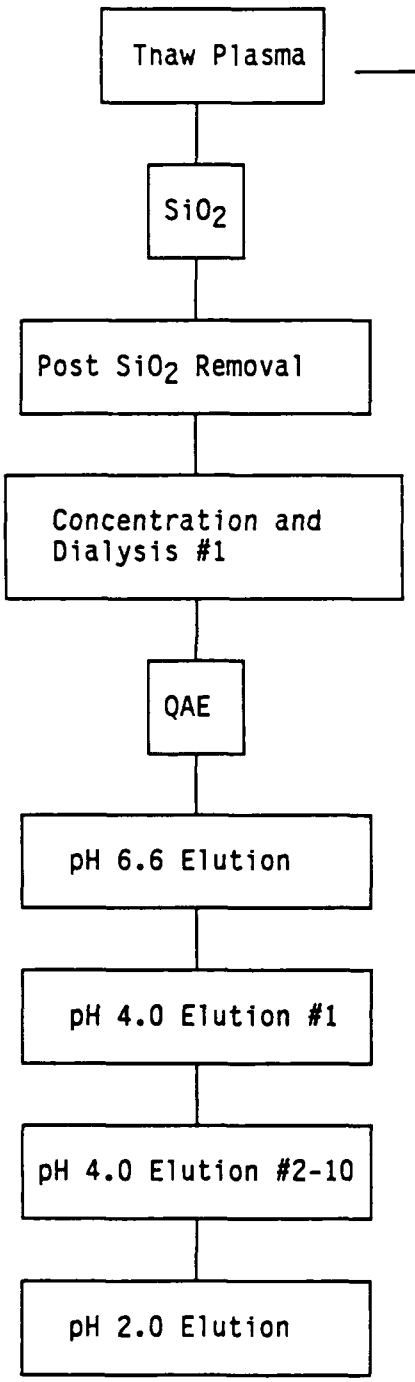
[1] Log_{10} of tissue culture infectious dose

[2] In this and in subsequent Flow Sheets, the "Fraction Surviving" = TCID-50 of an aliquot taken at a processing stage divided by the TCID-50 of an aliquot taken immediately after the previous spiking.

[3] NVR = no virus recovered (aliquot of pH 6.6 eluate was assayed starting with undiluted sample; all other aliquots were assayed starting at a 10^{-1} sample dilution.

FLOW SHEET EXPERIMENT #2

Negative plasma seeded with HTLV-III at start of processing



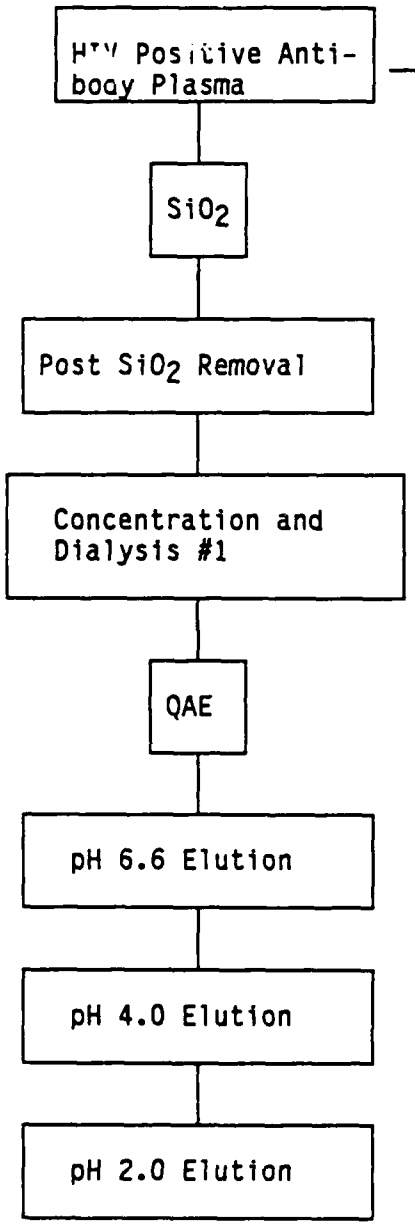
	TCID-50/ML[1]	Fraction Surviving
Thaw Plasma — HTLV-III	6.7	10^{0}
SiO_2	7.5	
Post SiO_2 Removal	5.2	$10^{-1.5}$
Concentration and Dialysis #1	4.4	$10^{-2.3}$
QAE		
pH 6.6 Elution	NVR[2]	$<10^{-6.7}$
pH 4.0 Elution #1	1.2	$10^{-5.5}$
pH 4.0 Elution #2-10	NVR	$<10^{-6.7}$
pH 2.0 Elution	NVR	$<10^{-6.7}$

[1] Log_{10} tissue culture infectious dose

[2] No virus recovered (pH of aliquots neutralized when necessary to allow all assays to start with an undiluted sample.)

FLOW SHEET EXPERIMENT #3

HIV positive plasma seeded with HTLV-III at start of processing.
Fluorescent titer 1:1 X 10^5



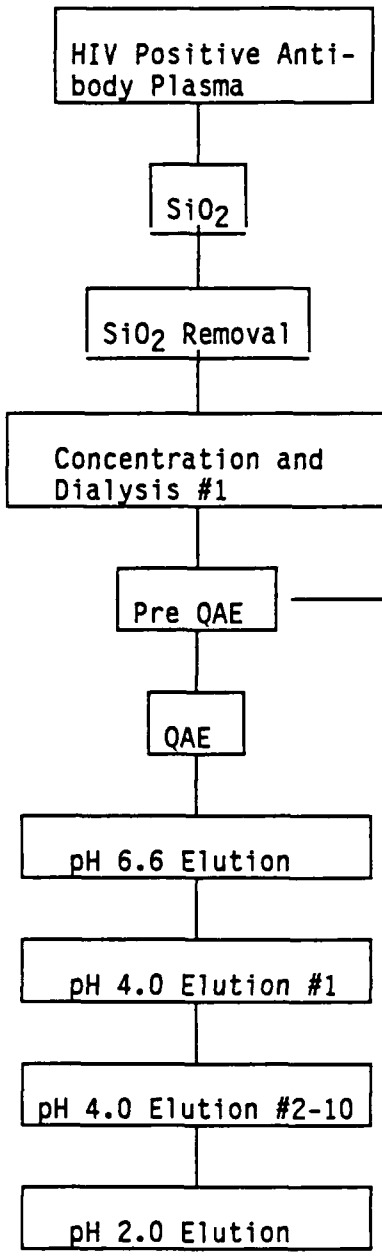
	TCID-50/ML[1]	Fraction Surviving
Pre-spike virus	8.7	---
HTLV-III spike	4.0	10^0
	4.2	
	2.7	$10^{-1.3}$
	1.5	$10^{-2.5}$
	NVR[2]	$<10^{-4}$
	NVR	$<10^{-3}$
	NVR	$<10^{-3}$

[1] Log_{10} tissue culture infectious dose

[2] No virus recovered (aliquot of pH 6.6 eluate was assayed starting with undiluted sample; all other aliquots were assayed starting at a 10^{-1} sample dilution).

FLOW SHEET EXPERIMENT #4

HIV positive plasma seeded with HTLV-III at start of processing and again just prior to column. Fluorescent titer 1:10,000



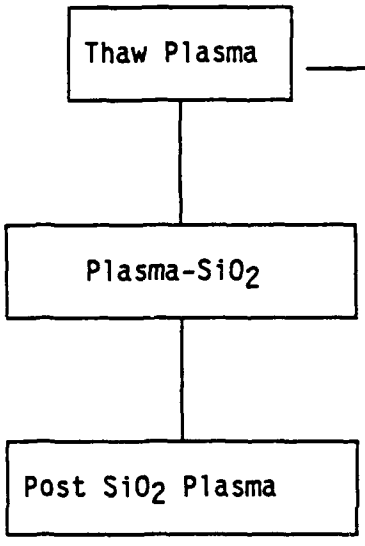
	TCID-50/ML[1]	Fraction Surviving
Pre-spike virus (#1)	9.0	---
HTLV-III spike	7.2	10^{0}
	6.7	$10^{-0.5}$
	4.2	$10^{-3.0}$
	3.4	$10^{-3.8}$
Pre-spike virus (#2)	8.2	---
HTLV-III spike	7.0	10^{0}
	NVR[2]	$<10^{-7}$
	0.7	$<10^{-6.3}$
	NVR	$<10^{-6}$
	NVR	$<10^{-6}$

[1] Log_{10} of tissue culture infectious dose

[2] No virus recovered (aliquot of pH 6.6 eluate was assayed starting with undiluted sample; all other aliquots were assayed starting at a 10^{-1} sample dilution).

FLOW SHEET EXPERIMENT #5

Negative plasma seeded and treated with wet SiO_2.

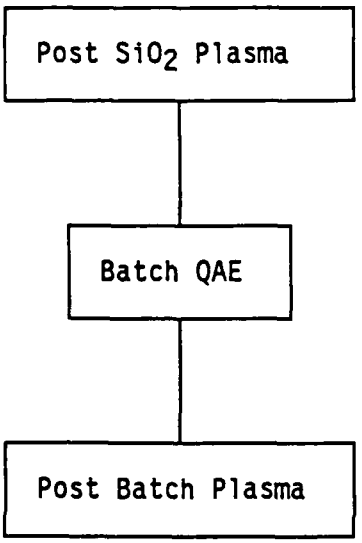


	TCID-50/ML[1]	Fraction Surviving
Thaw Plasma — HTLV-III	6.7	10^{0}
Plasma-SiO_2		
Post SiO_2 Plasma	5.2	$10^{-1.5}$

[1] Log_{10} of tissue culture infectious dose

FLOW SHEET EXPERIMENT #6

Negative plasma seeded and absorbed with QAE batch (wetted).

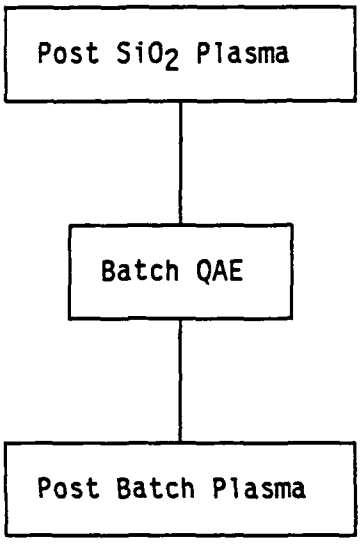


		TCID-50/ML[1]	Fraction Surviving
Post SiO_2 Plasma	HTLV-III spike	7.5	10^0
Post Batch Plasma		6.7	$10^{-0.8}$

[1] Log_{10} of Tissue culture infectious dose

FLOW SHEET EXPERIMENT #7

Negative plasma seeded and absorbed with QAE batch (dry).

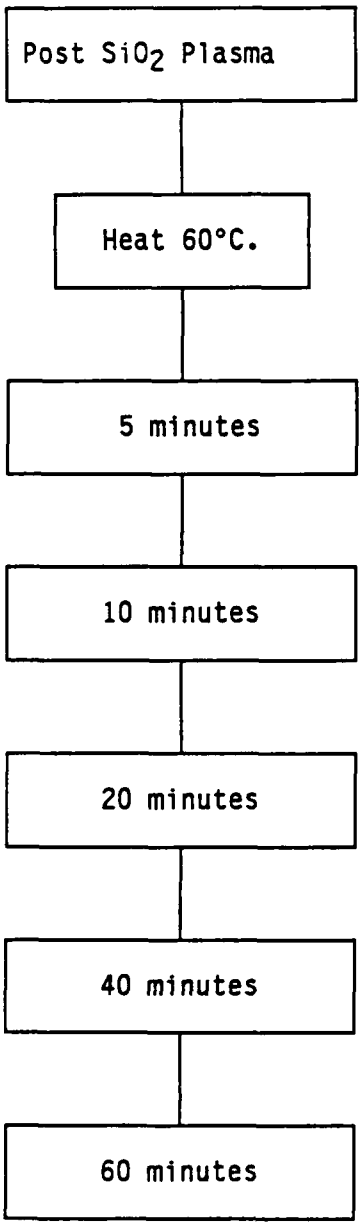


	TCID-50/ML[1]	Fraction Surviving
HTLV-III spike	7.4	10^{0}
	7.2	$10^{-0.2}$

[1] Log_{10} of Tissue culture infectious dose

FLOW SHEET EXPERIMENT #8

Negative plasma seeded with HTLV-III and heat inactivated.



	TCID-50/ML[1]	Fraction Surviving
Pre-spike virus	8.5	
HTLV-III spike	7.9	10^{0}
5 minutes	7.4	$10^{-0.5}$
10 minutes	4.7	$10^{-3.2}$
20 minutes	2.4	$10^{-5.5}$
40 minutes	NVR[2]	$<10^{-7.9}$
60 minutes	NVR	$<10^{-7.9}$

[1] Log_{10} of tissue culture infectious dose

[2] No virus recovered

FLOW SHEET EXPERIMENT #9

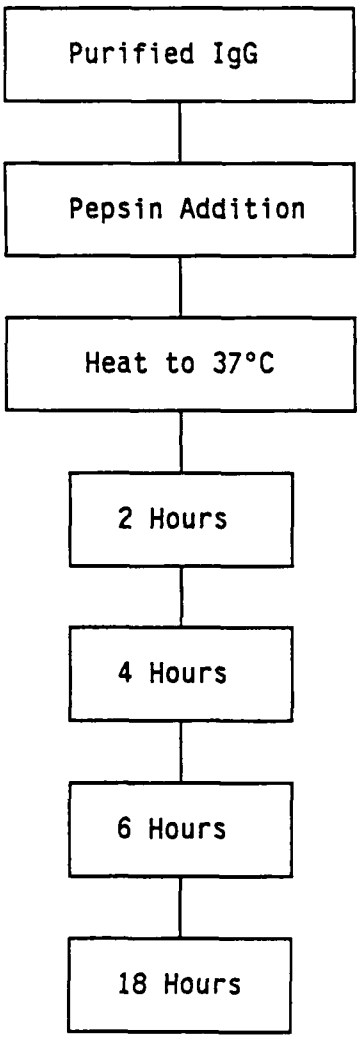
Negative plasma plus pepsin seeded with HTLV-III and heat inactivated

		TCID-50/ML[1]	Fraction Surviving
	Pre-spike virus	8.4	
Post SiO_2 Plasma	HTLV-III spike	7.7	10^0
Pepsin Addition			
Heat 60°C.			
5 minutes		6.2	$10^{-1.5}$
10 minutes		2.7	$10^{-5.0}$
20 minutes		1.7	$10^{-6.0}$
40 minutes		1.2	$10^{-6.5}$
60 minutes		2.2	$10^{-5.5}$

[1] Log_{10} of tissue culture infectious dose

FLOW SHEET EXPERIMENT #10

Purified IgG plus pepsin seeded with HTLV-III and held at 37°C.



	TCID-50/ML[1]	Fraction Surviving
Pre-spike	8.2	
HTLV-III Spike	7.0	10^{0}
2 Hours	4.0	$10^{-3.0}$
4 Hours	0.7	$10^{-6.3}$
6 Hours	2.0	$10^{-5.0}$
18 Hours	NVR[2]	$<10^{-7.0}$

[1] Log_{10} of tissue culture infectious dose

[2] No virus recovered

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Table I

Human Anti-Lassa Fever Final Product

Pool Two Yields

Final Product	Initial Protein (mg)	Final Protein (mg)	Gm/Liter Yield	% Yield	Final Conc (mg/ml)	No. of Vials*
Intact $IgG_{1,2,4}$ (QAE)	80,328	12,300	9.72	15.3	24.6	46
Intact $IgG_{1,2,3,4}$ (CM)	77,418	13,516	10.68	17.4	24.8	53

* Bottled in 10cc vials

Table II

Protection of Cynomolgus Monkeys Against Lassa Virus

(Liberian Strain Z-158) by ALF-2

Treatment	LNI	Dose*	Dead/Total	Viremia-log_{10} Day 7	pfu/ml Day 14
Plasma	1.6	6	3/4	<	4.6
		3	2/2	2.3	4.7
Intact $IgG_{1,2,4}$ (QAE)	3.0	6	0/3	<	<
Intact $IgG_{1,2,3,4}$ (CM)	3.5	6	0/2	<	<
None	-	-	4/4	3.5	4.0

* ml/kg inoculated I.V. on days 0,3 & 6

Table III

Protection of Guinea Pigs Against Lassa Virus

(Liberian Strain Z-132) by ALF-2

Treatment	LNI	Dose[a]	Dead/5	MTD[b]	Viremia-$\log_{10}$ Day 7	pfu/ml Day 14
Plasma	1.6	6	3	28.0	<	2.0
		3	5	18.2	2.2	4.3
Intact $IgG_{1,2,4}$ (QAE)	3.0	6	0	--	<	<
		3	1	29.0	<	<
		1	5	21.2	<	3.0
Intact $IgG_{1,2,3,4}$ (CM)	3.5	6	0	--	<	<
		3	0	--	<	3.1
None	-	-	5	18.4	3.3	3.6

[a] ml/kg inoculated I.P. on days 0,3 & 6
[b] Mean time of death

Table IV

Human ALF-3 Final Product Yields

Final Product	Initial Protein (mg)	Final Protein (mg)	Gm/Liter Yield	% Yield	Final Conc (mg/ml)	No. of Vials*
Intact $IgG_{1,2,4}$ (QAE)	205,466	36,608	9.63	17.8	25.6	69
Intact $IgG_{1,2,3,4}$ (CM)	238,830	36,579	9.63	15.3	25.8	70
$F(ab')_2$	214,434	20,632	5.43	9.6	25.8	40
Intact IgG (Cohn)	222,615	26,948	7.05	12.1	25.0	41
Intact IgG (Cohn Deaggregated)	--	--	--	--	25.1	13
Post SiO_2	--	552	--	--	27.6	1

* Bottled in 20cc vials

Table V

Protective Efficacy of ALF-3 For Guinea Pigs Against Human Lassa Virus, Z-132, Liberia

Treatment	IFAT[a]	LNI[b]	Dose[c] (ml/kg)	Results of Challenge % Protected (n=5)	MTD[d]
Unfractionated Plasma	640	1.9	3	40	21.0
$F(ab')_2$	1280	1.0	3	0	21.0
			1.5	0	20.0
Intact $IgG_{1,2,4}$ (QAE)	1280	2.8	3	80	20.0
			1.5	60	20.5
Intact $IgG_{1,2,3,4}$ (CM)	1280	3.2	3	100	---
			1.5	80	22.3
Intact IgG (Cohn)	1280	2.6	3	100	---
Intact IgG (Cohn Deaggregated)	---	3.4	3	100	---
None	---	---	---	0	20.5

[a] Indirect fluorescent antibody titer
[b] Log neutralizing index
[c] Immune globulin administered I.P. on days 0,3 and 6
[d] Mean time of death

Table VI

Protective Efficacy of ALF-3 For Guinea Pigs Against Lassa Virus,

Josiah Strain, Sierra Leone

Treatment	IFAT[a]	LNI[b]	Dose[c] (ml/kg)	Results of Challenge % Protected (n=5)	MTD[d]
Unfractionated Plasma	640	1.6	3	40	19.6
			1.5	20	19.0
$F(ab')_2$	1280	0.6	3	0	19.6
			1.5	0	20.2
Intact $IgG_{1,2,4}$ (QAE)	1280	2.6	3	80	23.0
			1.5	20	20.0
Intact $IgG_{1,2,3,4}$ (CM)	1280	2.8	3	60	17.5
			1.5	60	22.0
Intact IgG (Cohn)	1280	2.0	3	80	19.0
Intact IgG (Cohn Deaggregated)	1280	2.1	3	100	---
None	---	---	---	0	21.6

[a] Indirect fluorescent antibody titer
[b] Log neutralizing index
[c] Immune globulin administered I.P. on days 0,3 and 6
[d] Mean time of death

Table VII

Efficacy of ALF-3, Ribavirin, or Combined Therapy in Treatment of Guinea Pigs Infected With Lassa Virus, Josiah Strain

Treatment[a]	LNI[b]	Ribavirin[c] 20 mg/kg d0-10	Dead/ Total	MTD[d]	Viremia (log_{10} PFU/ml) d7	d14	d20
---	---	+	4/5	21.0	<	2.28	3.02
Intact $IgG_{1,2,4}$ (QAE)	2.8	---	4/5	20.0	<	2.83	3.92
		+	0/5	---	<	1.32	1.64
Intact $IgG_{1,2,3,4}$ (CM)	3.3	---	2/5	22.0	<	1.84	1.55
		+	0/5	---	<	1.02	1.21
$F(ab')_2$	1.1	---	5/5	19.4	2.84	3.54	2.82
		+	5/5	22.0	<	2.10	2.92
---	---	---	5/5	18.2	3.34	3.83	(dead)

[a] 1.5 ml/kg dose on days 0,3 & 6
[b] Log neutralizing Index
[c] Ribavirin dose divided by 2 and inoculated at 12-hour intervals I.P.
[d] Mean time of death

Table VIII

Monkey Final Product Yields

Final Product	Initial Protein (mg)	Final Protein (mg)	Gm/Liter Yield	% Yield	Final Conc (mg/ml)	No. of Vials*
Intact IgG	69,497	7,229	7.86	10.4	25.1	28
$F(ab')_2$	69,175	4,652	5.06	6.7	25.7	18
$F(ab')_2$ Tailings	--	42	--	.9	4.7	9

* Bottled in 10cc vials

Table IX

Human Argentinian Hemorrhagic Fever Final Product Yields

Final Product	Initial Protein (mg)	Final Protein (mg)	Gm/Liter Yield	% Yield	Final Conc (mg/ml)	No. of Vials*
Intact $IgG_{1,2,4}$ (QAE)	125,767	10,838	4.73	8.6	25.5	22
Intact $IgG_{1,2,3,4}$ (CM)	126,706	12,182	5.32	10.0	25.4	25
$F(ab')_2$	120,156	7,348	3.21	6.1	25.5	14

* Bottled in 20cc vials

Table X

Passive Protection of Junin Virus-Infected Guinea Pigs by AHF-1

Treatment	1/PRN(80%) Titer	TU[a]/ Guinea Pig	# Dead/ Total	# Paralyzed/ Total	MTD[b] (Range)
Plasma	64	6000[c]	5/10	3/10	28.6(25-31)
Intact $IgG_{1,2,4}$ (QAE)	128	6000	0/5	0/5	--
Intact $IgG_{1,2,3,4}$ (CM)	128	6000	0/5	0/5	--
$F(ab')_2$	128	6000	5/5	0/5	15.4(14-16)
Plasma	64	30,000[d]	3/6	2/6	22.3(21-24)
$F(ab')_2$	128	30,000	6/6	0/6	16.3(15-20)
None	--	--	10/10	0/10	15.3(14-20)

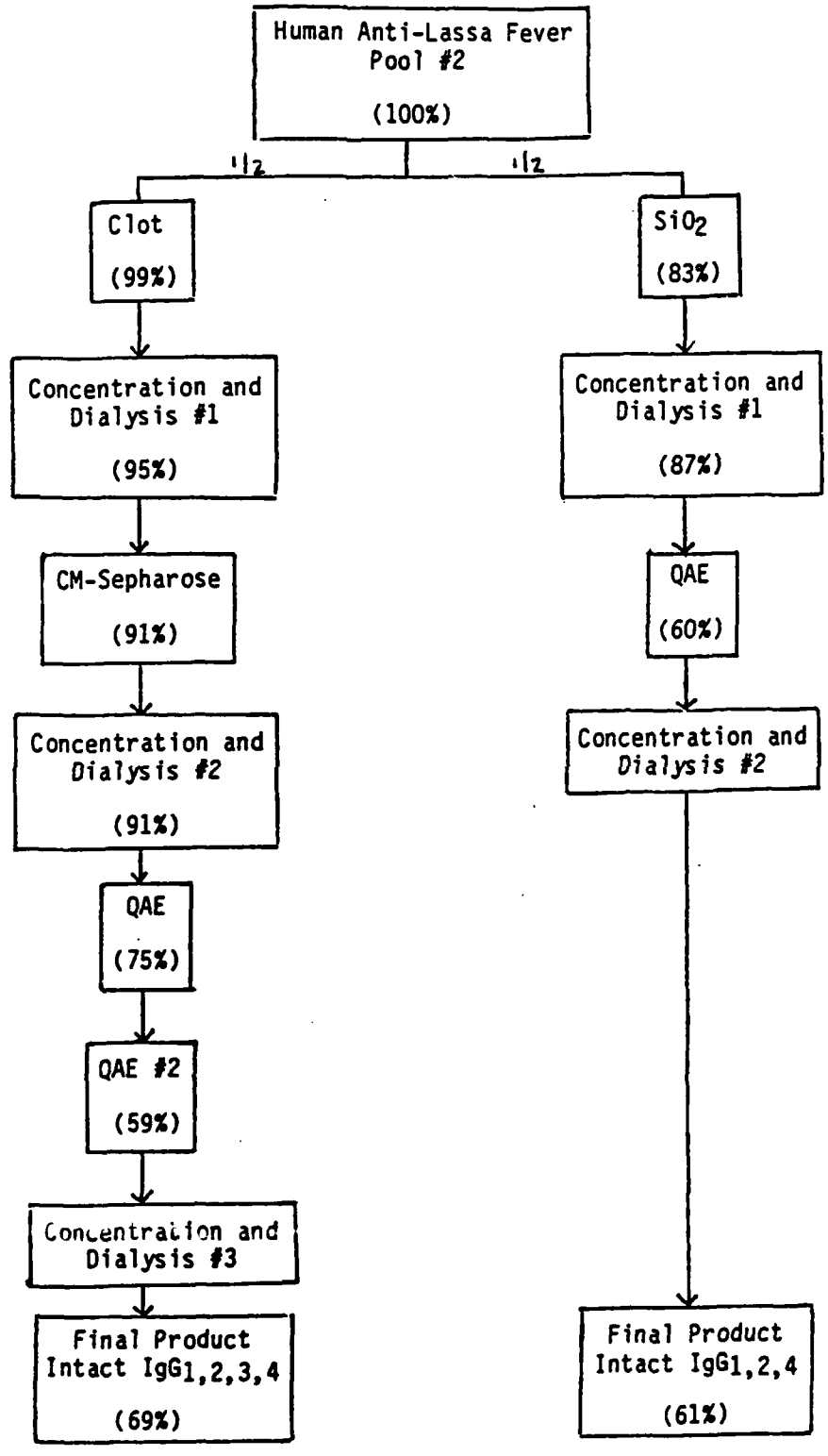
[a] TU (Therapeutic Units) = $\frac{\text{ml antiserum x 1/PRN titer}}{\text{body weight (kg)}}$

[b] Mean time of death

[c] Given 4 hours post infection

[d] 15,000 TU given 4 hour post infection and 15,000 TU given 6 days post infection

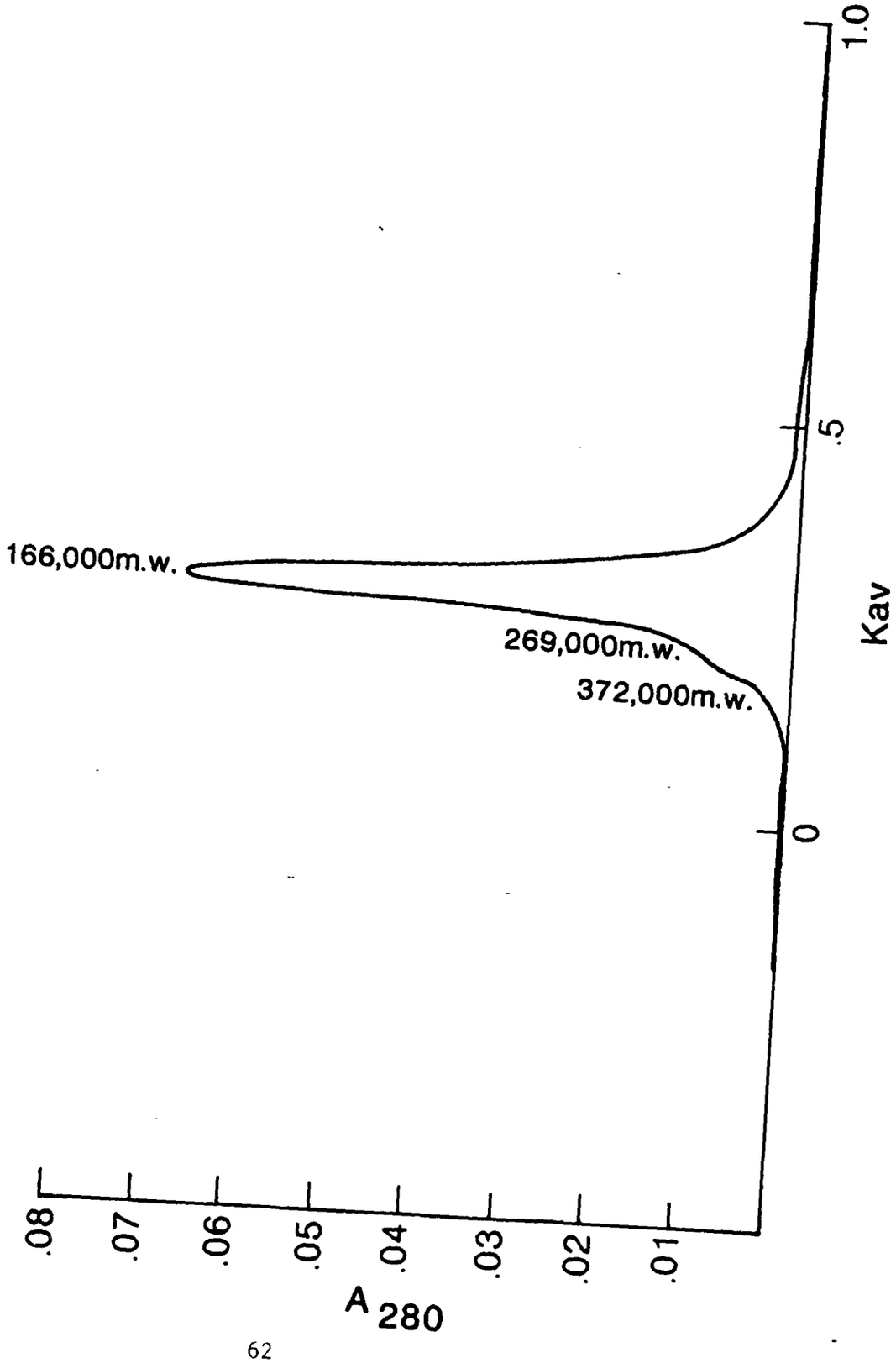
Figure 1
FRACTIONATION FLOW SHEET



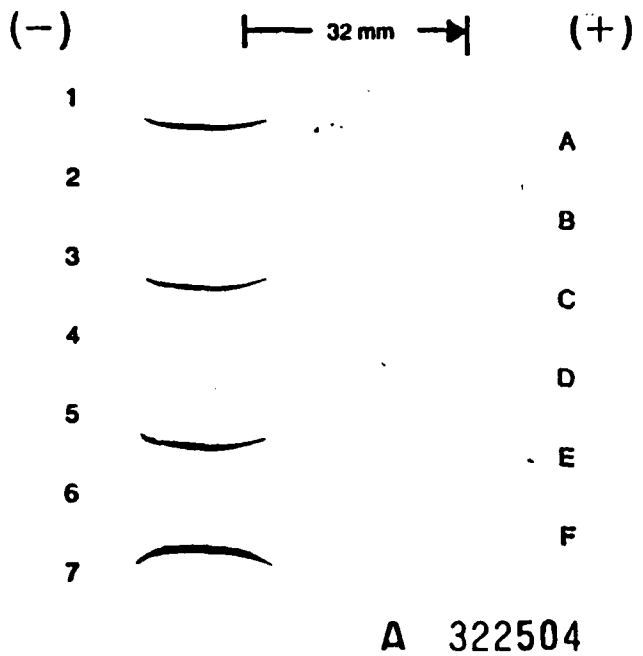
() Indicates IgG Yield

Figure 2

HPLC Human ALF-2 (CM) F.P. IgG



Immunoelectrophoresis of Human ALF-2(CM)



Samples Electrophoresed for 120 Minutes At 120 Volts
Antisera: A, C, E and F; Rabbit Anti Whole Human

Figure 3

Figure 4

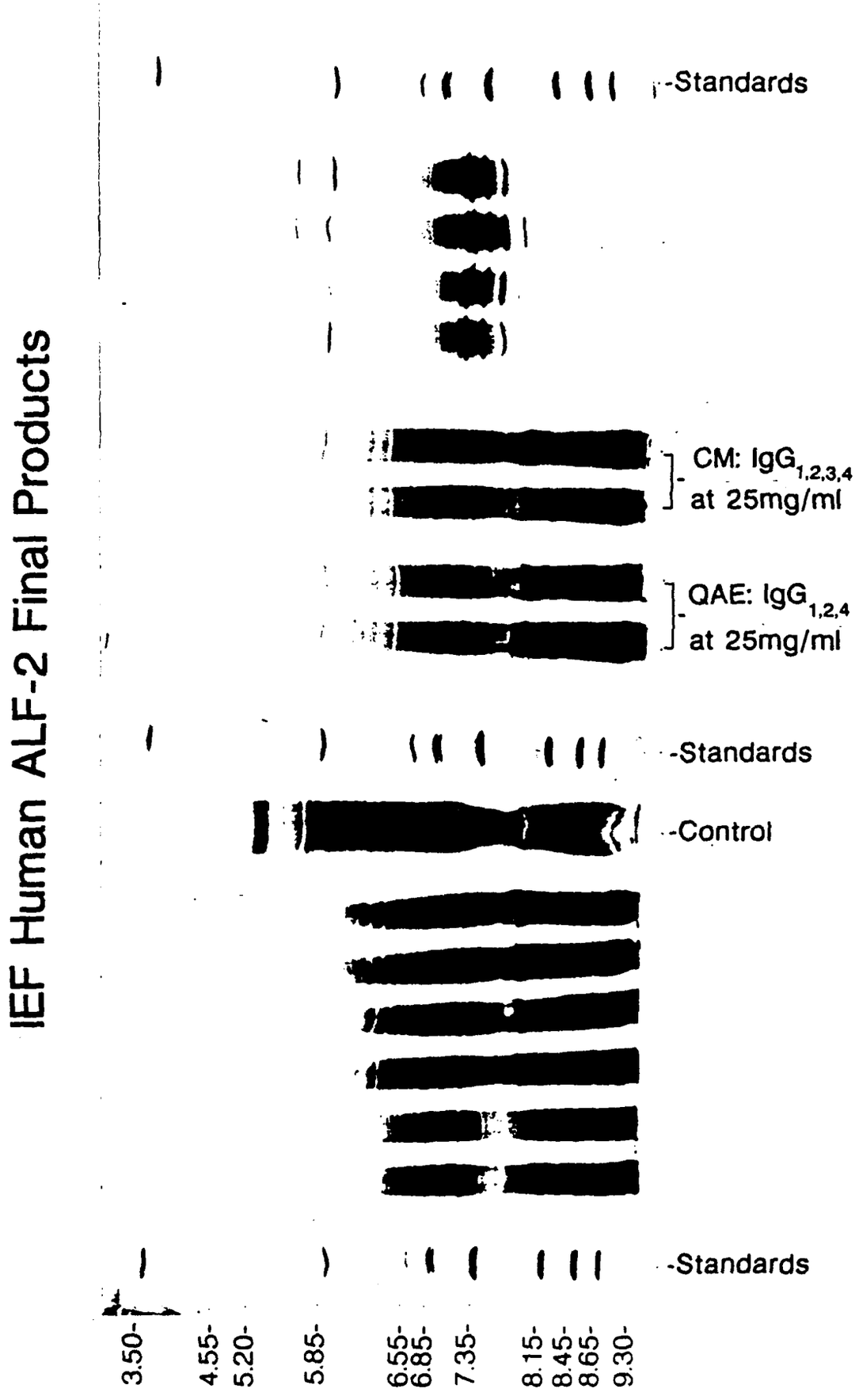
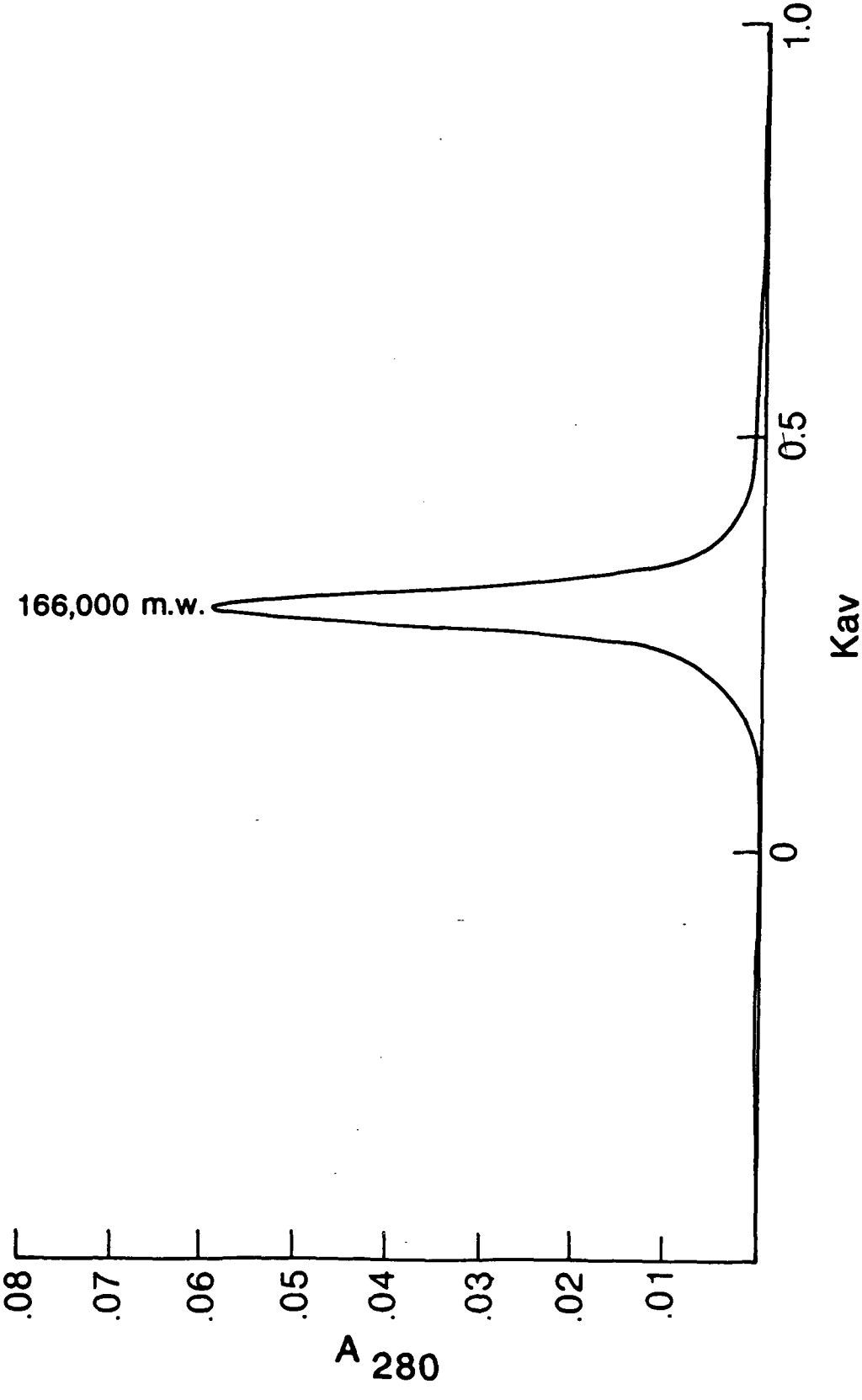
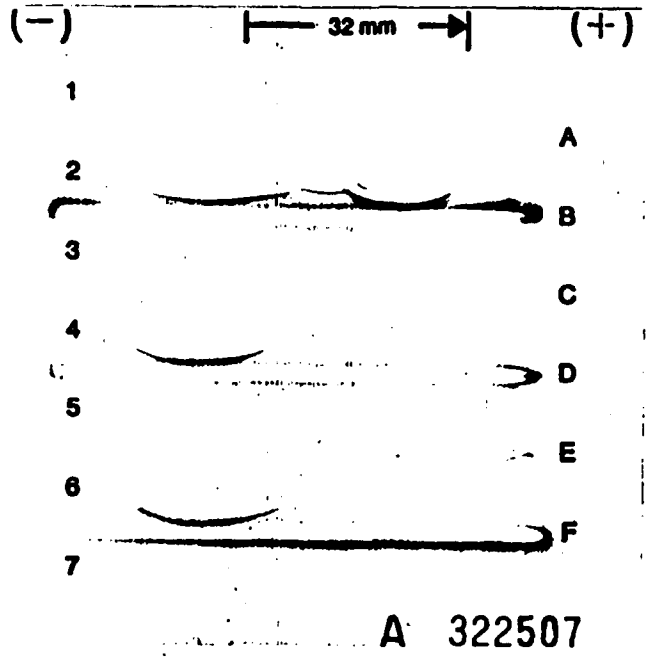

IEF Human ALF-2 Final Products
-Standards
CM: IgG1,2,3,4 at 25mg/ml
QAE: IgG1,2,4 at 25mg/ml
-Standards
-Control
-Standards
3.50-
4.55-
5.20-
5.85-
6.55-
6.85-
7.35-
8.15-
8.45-
8.65-
9.30-

Figure 5

HPLC Human ALF-2 (QAE) F.P. IgG



Immunoelectrophoresis of Human ALF-2(QAE)



1
2 Pre QAE
3
4 Post QAE
5
6 Final Product
7

Figure 6

Samples Electrophoresed for 120 Minutes At 120 Volts
Antisera: B,D, and F; Rabbit Anti Whole Human

Figure 7

FRACTIONATION FLOW SHEET

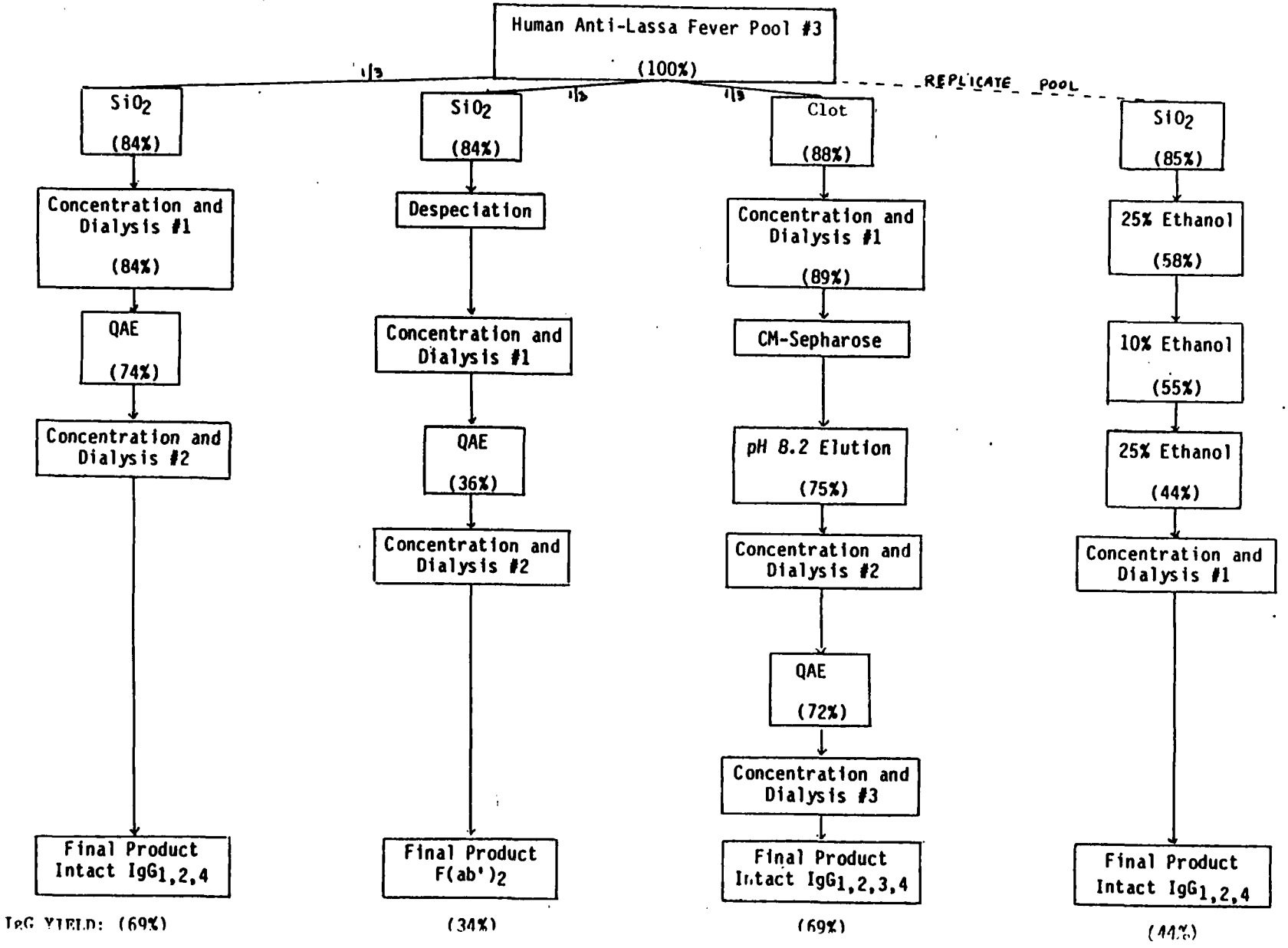
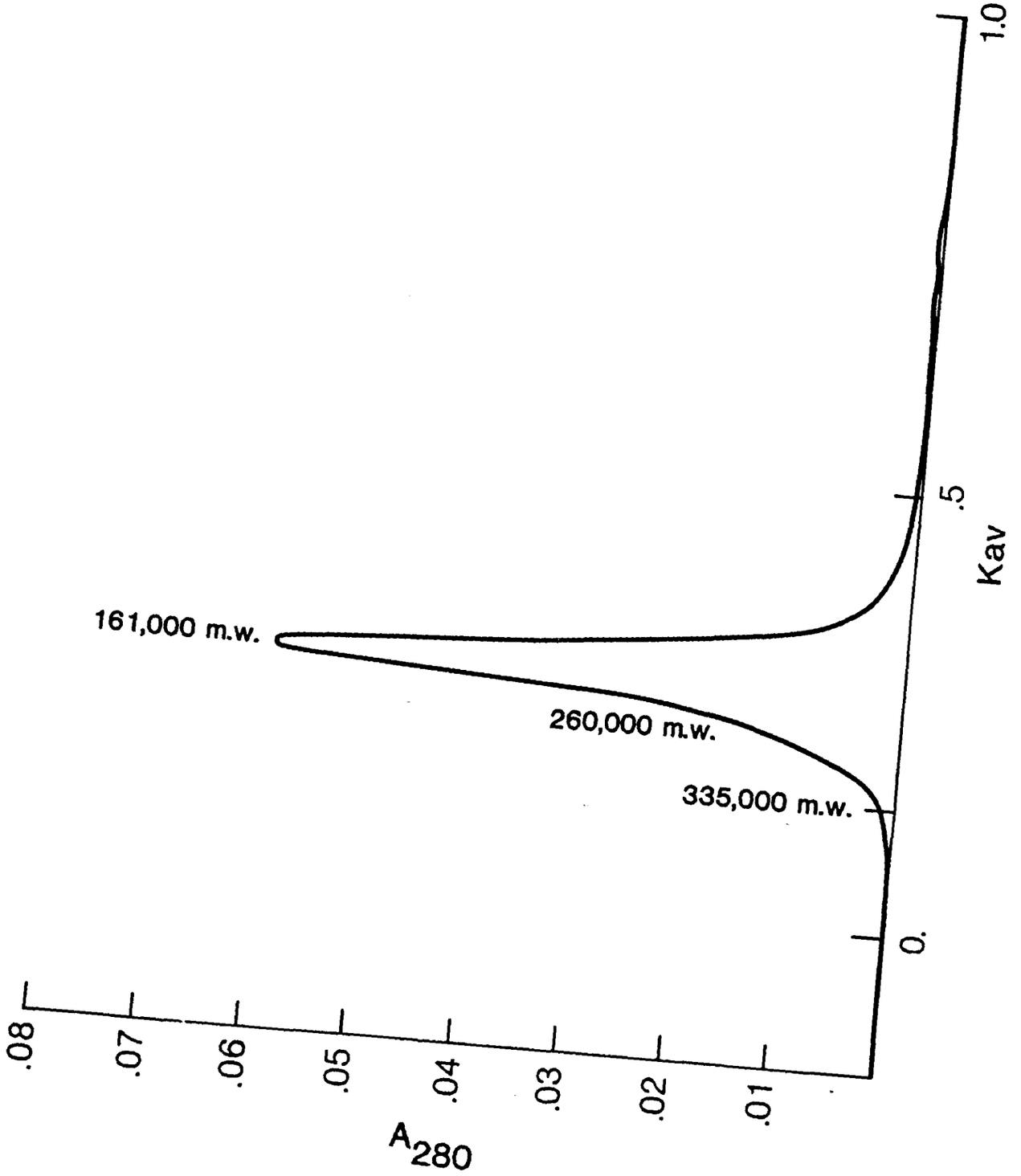
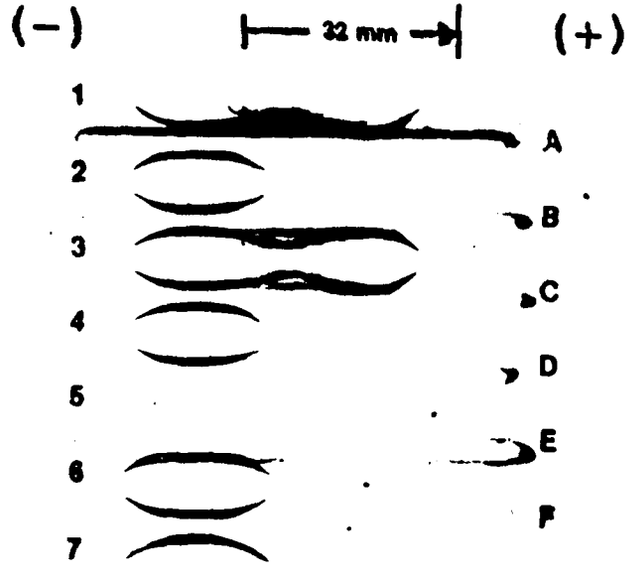


Figure 8

HPLC Human ALF-3(CM) F.P. IgG



Immunoelectrophoresis of Human ALF-3 (CM)



1 Pre QAE A

2 Post QAE A

3 Pre QAE B

4 Post QAE B

5

6 Final Product

7 Final Product

Figure 9

Samples Electrophoresed For 120 Minutes At 120 Volts
Antisera: A–E; Rabbit Anti Whole Human, F; Rabbit Anti Human IgG

Figure 10

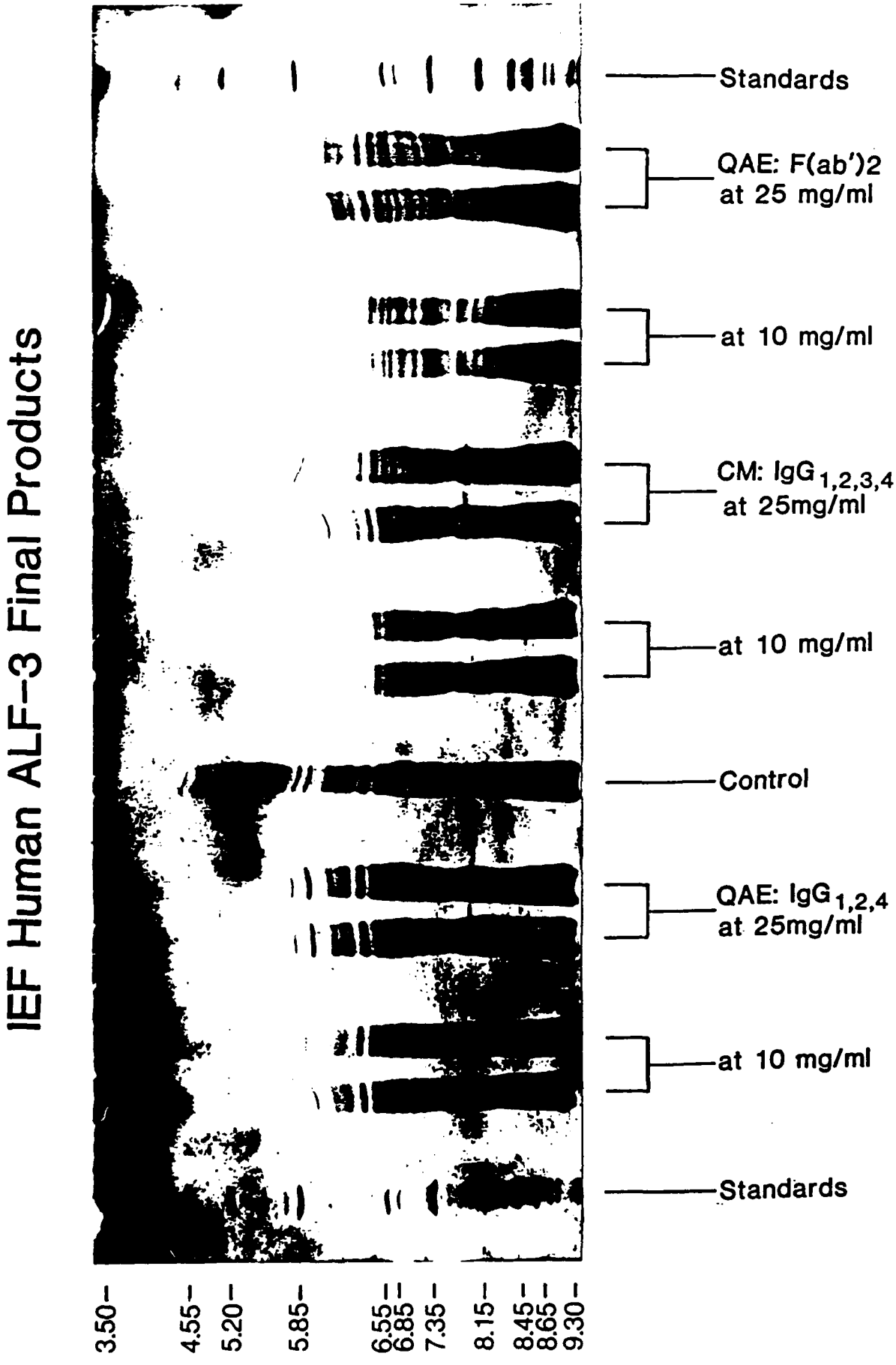
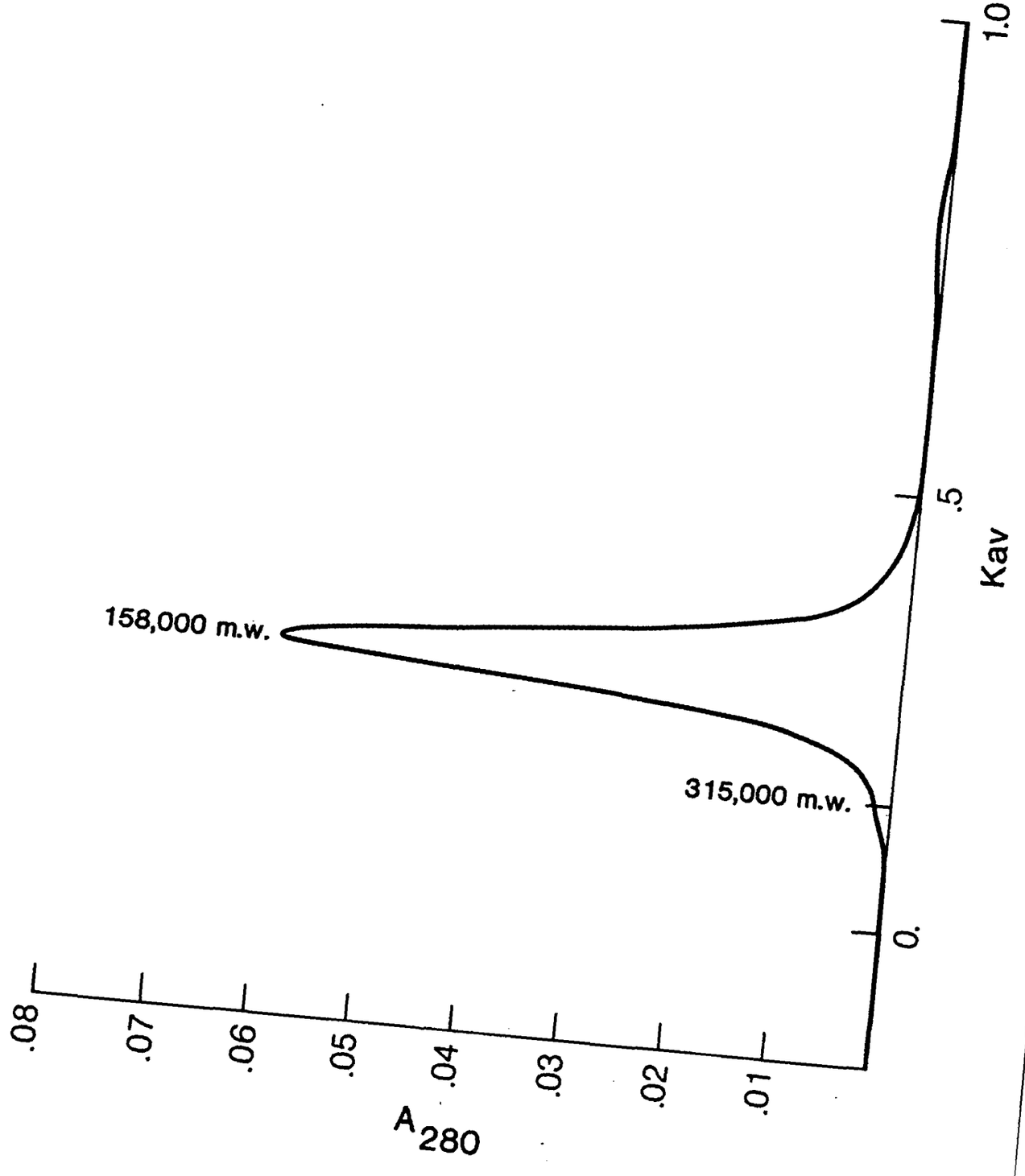
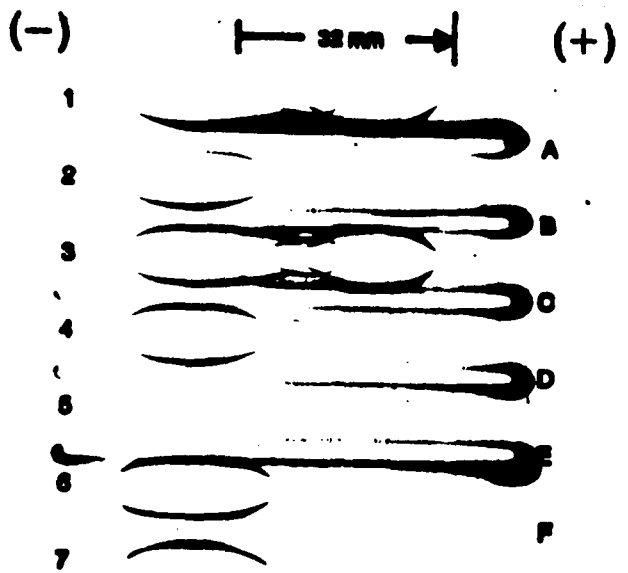

IEF Human ALF-3 Final Products
Standards
QAE: F(ab')2 at 25 mg/ml
at 10 mg/ml
CM: IgG1,2,3,4 at 25mg/ml
at 10 mg/ml
Control
QAE: IgG1,2,4 at 25mg/ml
at 10 mg/ml
Standards
3.50
4.55
5.20
5.85
6.55
6.85
7.35
8.15
8.45
8.65
9.30

Figure 11

HPLC Human ALF-3(QAE) F.P. IgG



Immunoelectrophoresis of Human ALF-3 (QAE)



1 Pre QAE A

2 Post QAE A

3 Pre QAE B

4 Post QAE B

5

6 Final Product

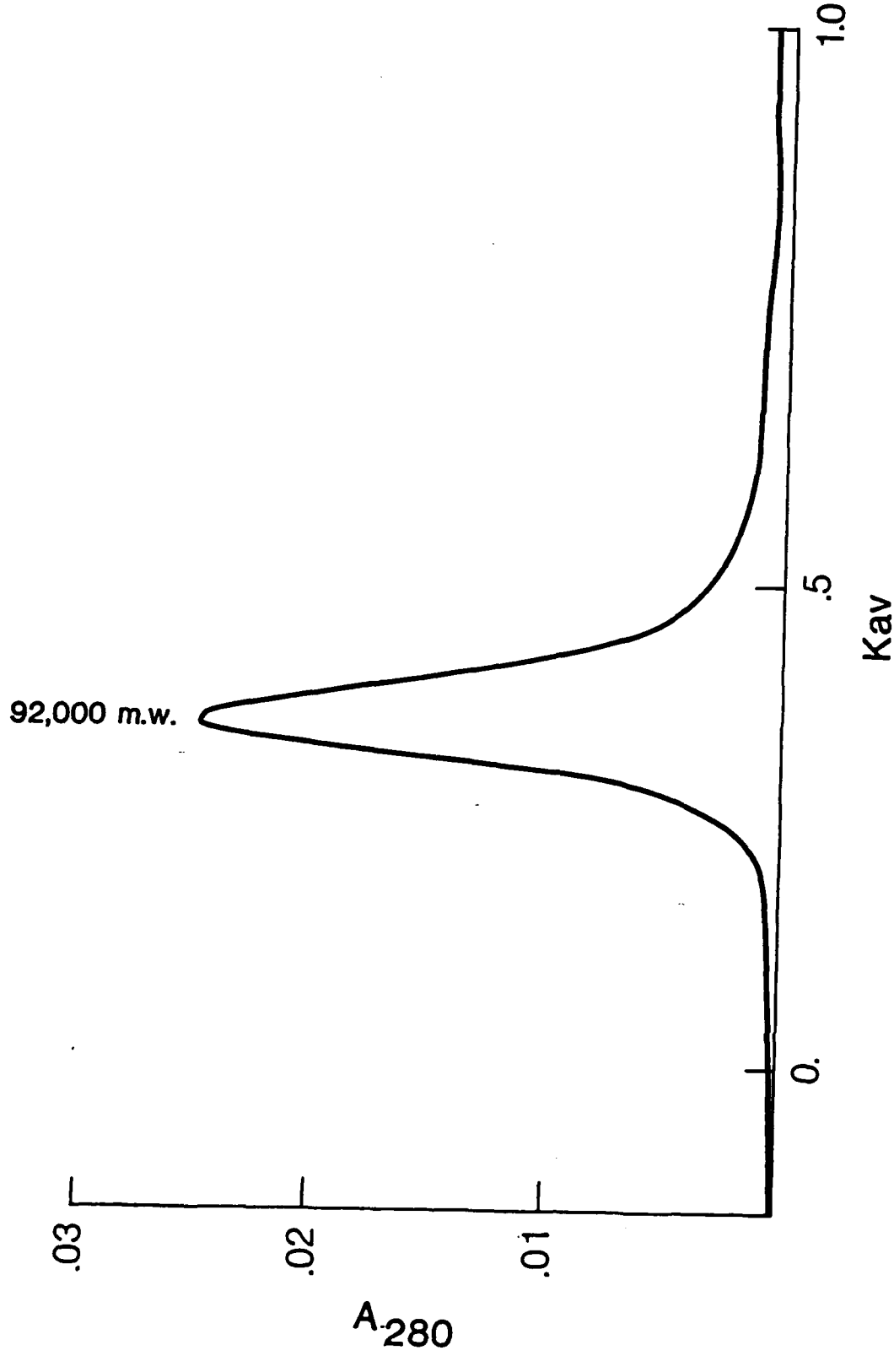
7 Final Product

Figure 12

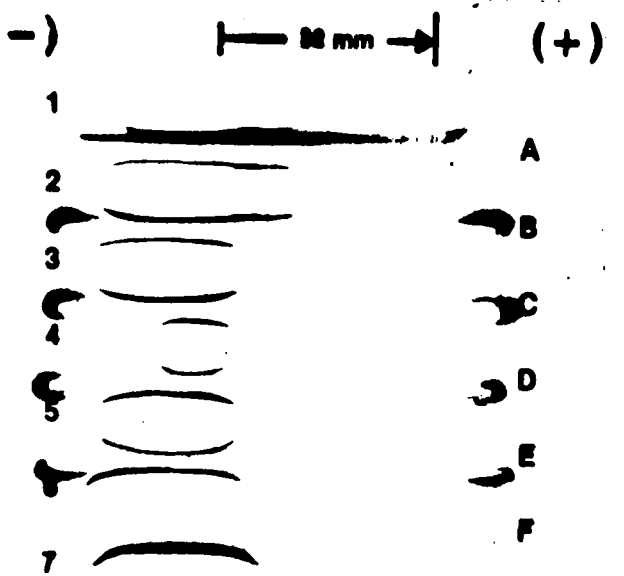
Samples Electrophoresed For 120 Minutes At 120 Volts
Antisera:A–E; Rabbit Anti Whole Human, F; Rabbit Anti Human IgG

Figure 13

HPLC Human ALF-3 F.P. $F(ab')_2$



Immunoelectrophoresis of Human ALF-3 F(ab')$_2$



1 Post Batch

2 Pre QAE

3 Post QAE Pool 1

4 Post QAE Pool 2

5 Post QAE Pools A+B

6 Final Product As Is

7 Final Product Conc. x2

Figure 14

Samples Electrophoresed For 120 Minutes At 120 Volts
Antisera: Gibco, Rabbit Anti Whole Human

Figure 15

HPLC Human ALF–3 (COHN) F.P. IgG

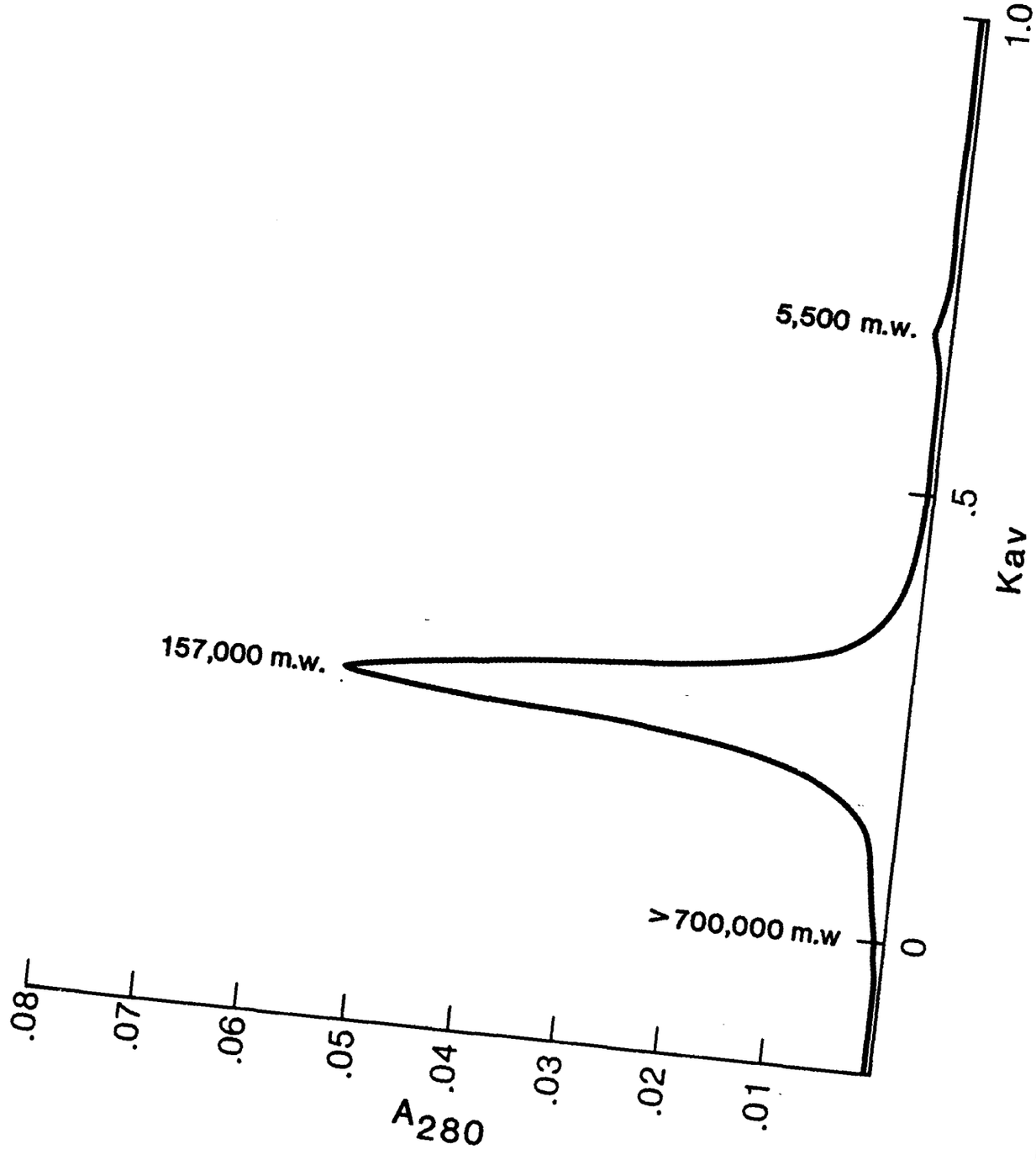
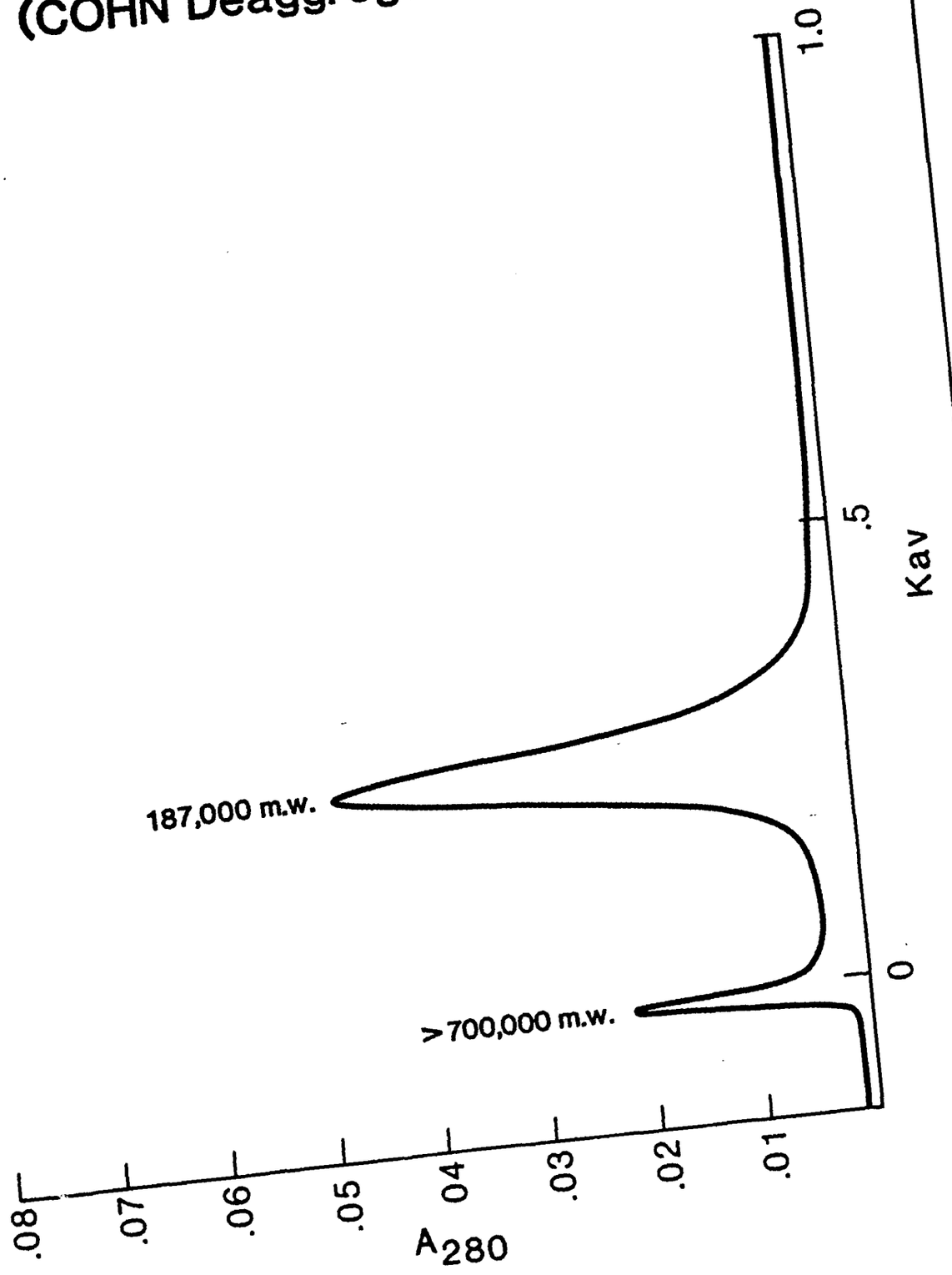
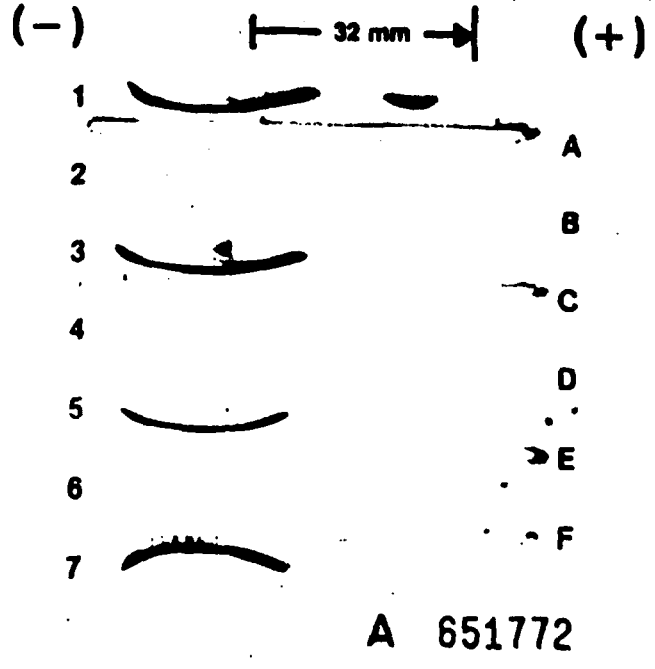


Figure 16

HPLC Human ALF–3 (COHN Deaggregated) F.P. IgG



Immunoelectrophoresis of Human ALF-3 (COHN)



1 Final Product (COHN)

2

3 Final Product (COHN Deaggregated)

4

5 Hyland I.M.

6

7 Cutter I.M.

Figure 17

Samples Electrophoresed For 120 Minutes At 120 Volts
Antisera: Gibco, Rabbit Anti Whole Human

Figure 18

FRACTIONATION FLOW SHEET

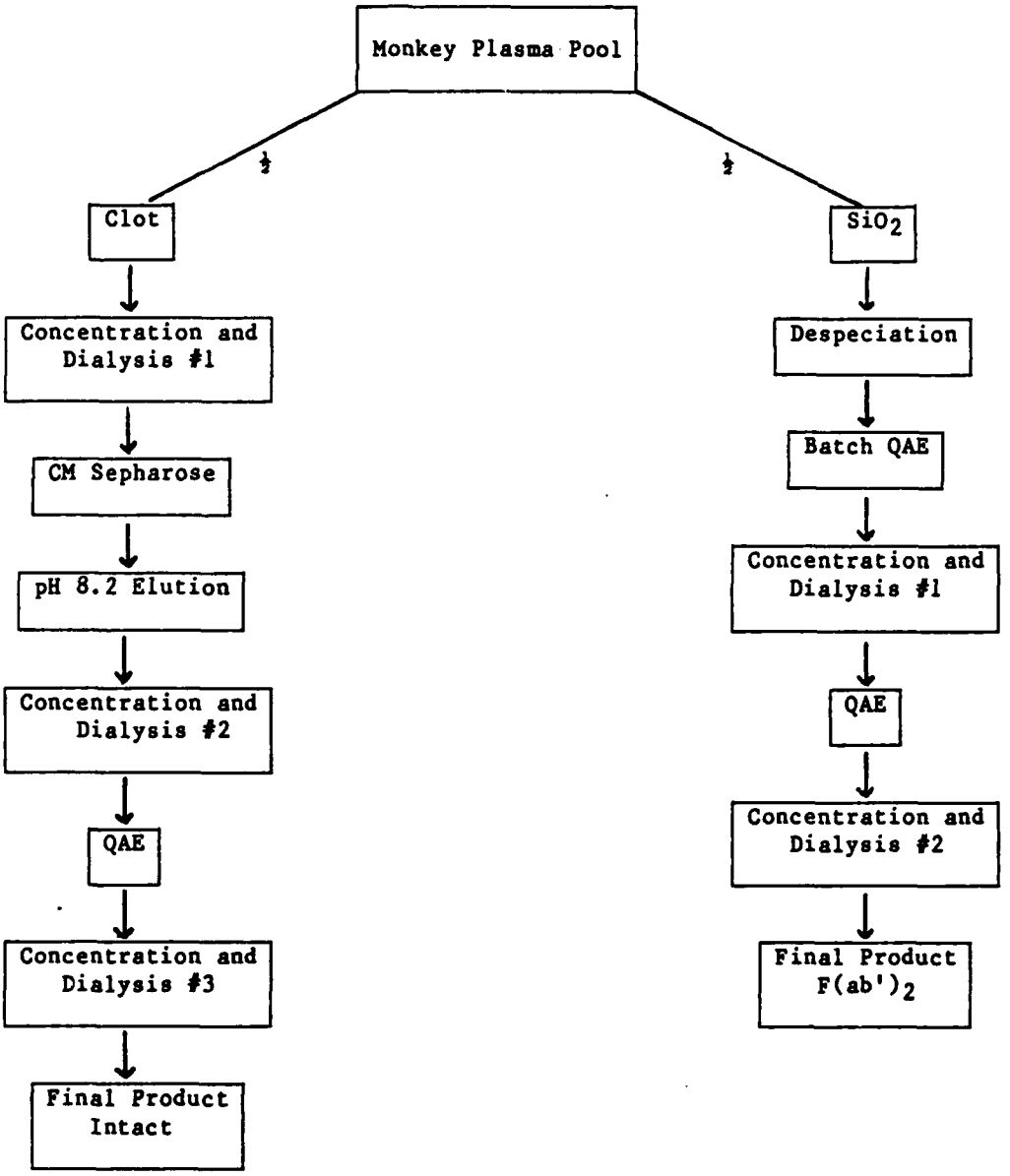
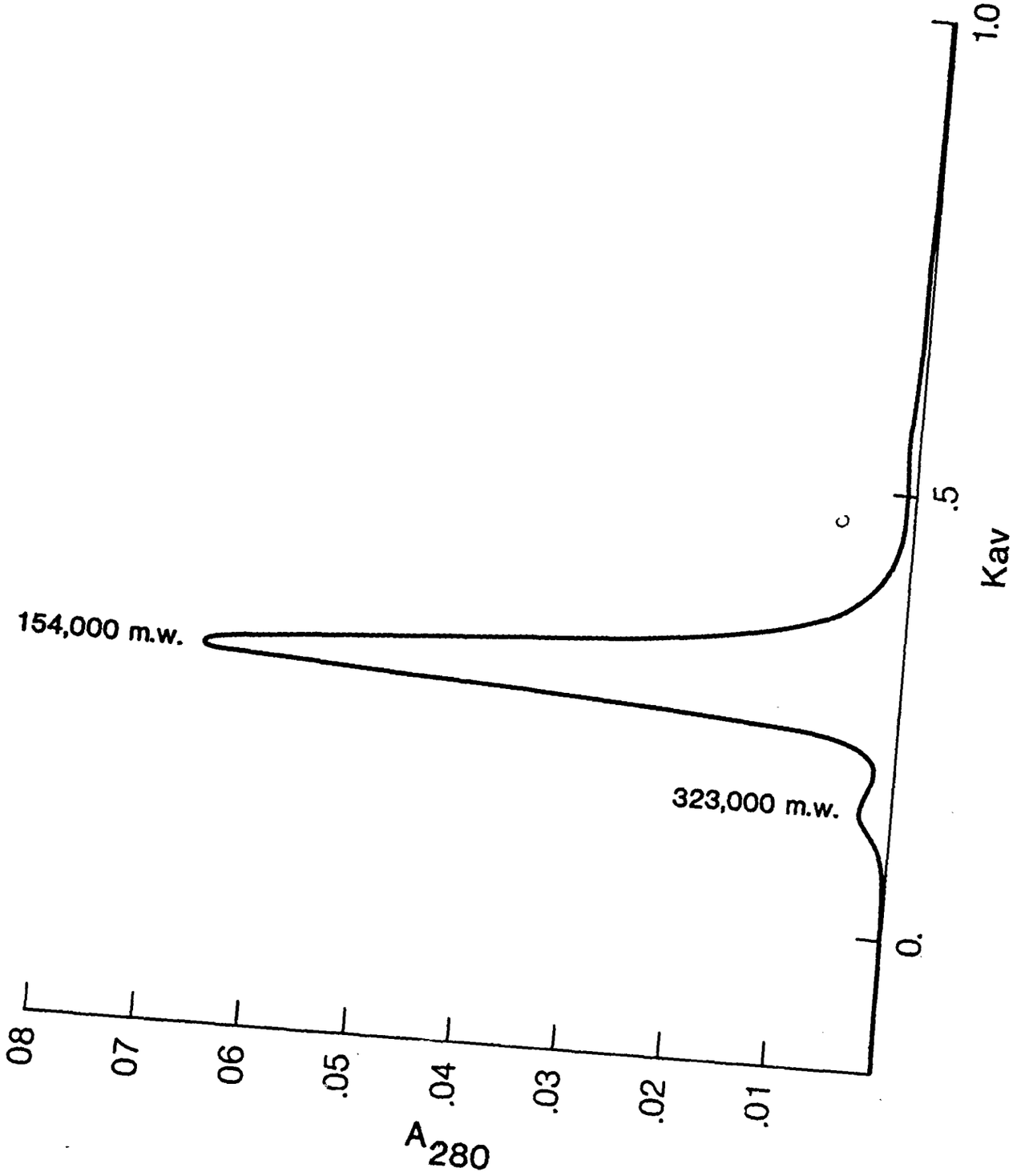
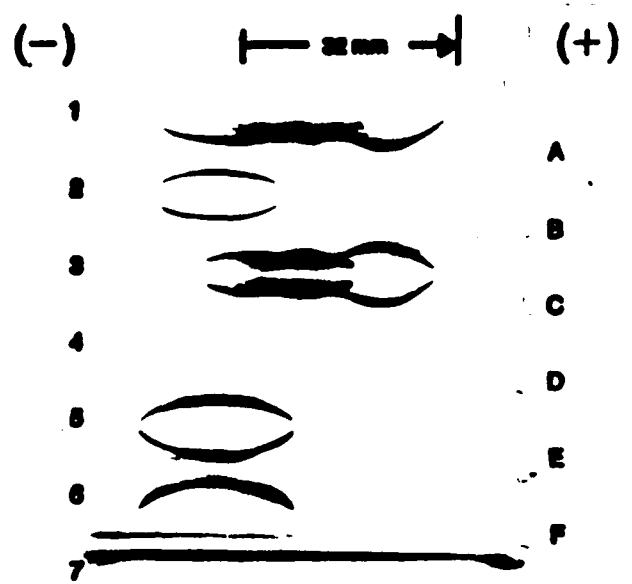


Figure 19

HPLC Monkey ALF–2 F.P. IgG



Immunoelectrophoresis of Monkey ALF-3



1 Pre QAE

2 Post QAE

3 pH 4.0 Elution

4

5 Final Product

6 Final Product

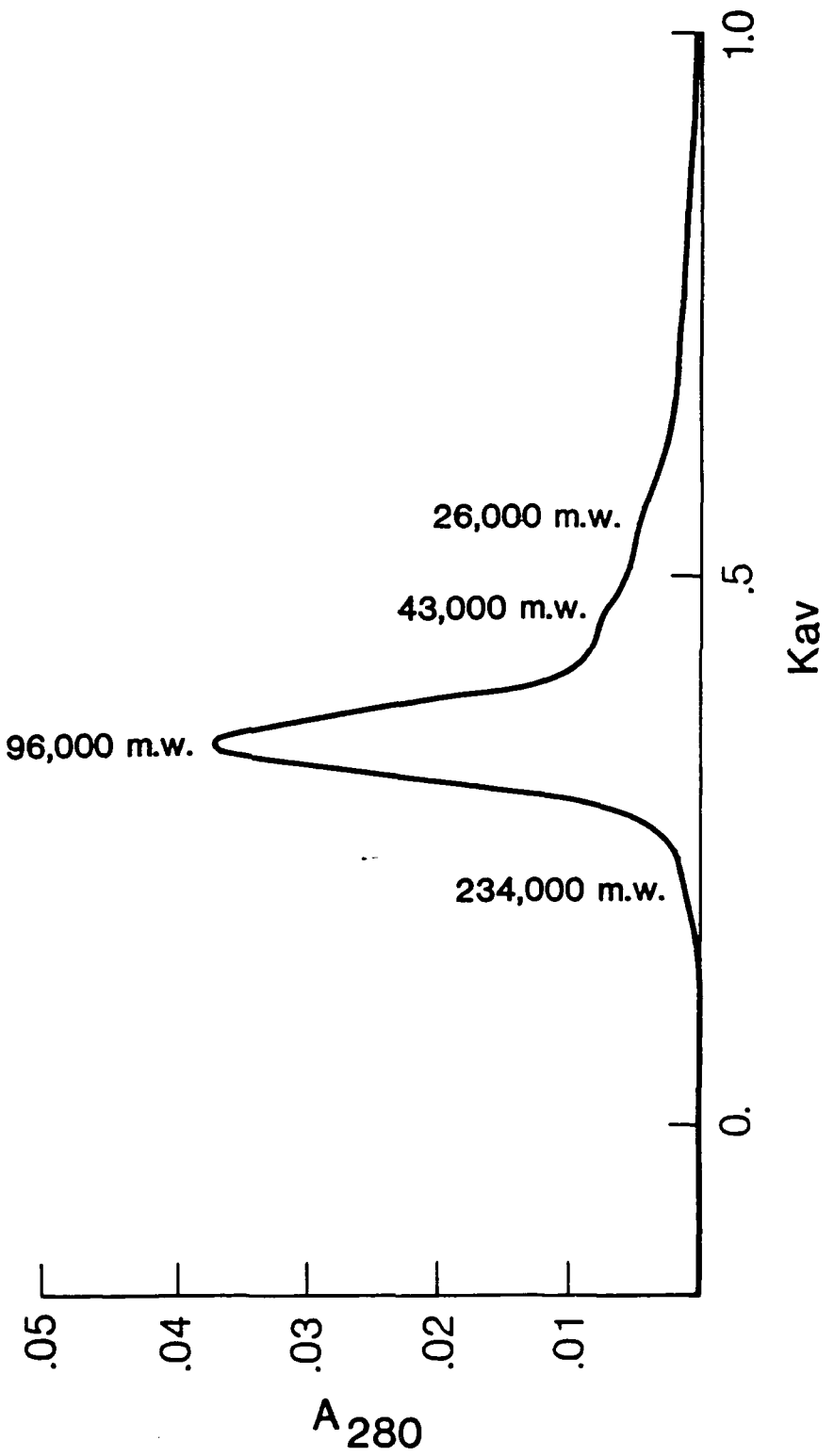
7 Final Product

Samples Electrophoresed For 120 Minutes At 120 Volts
Antisera: A–D; Anti Whole Monkey, E; Anti Monkey IgG, F; Anti Human Hemoglobin

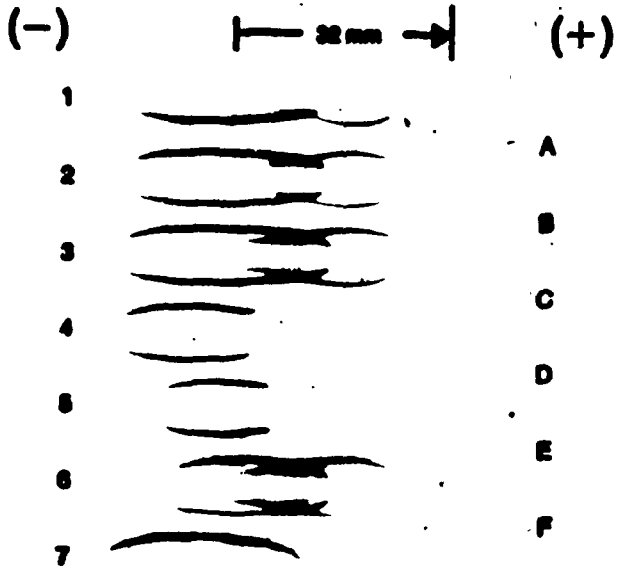
Figure 20

Figure 21

HPLC Monkey ALF-2 F.P. $F(ab')_2$



Immunoelectrophoresis of Monkey ALF-2 $F(ab')_2$



1 Pre Batch QAE

2 Post Batch QAE

3 Pre QAE

4 Post QAE Pool 1

5 Post QAE Pool 2

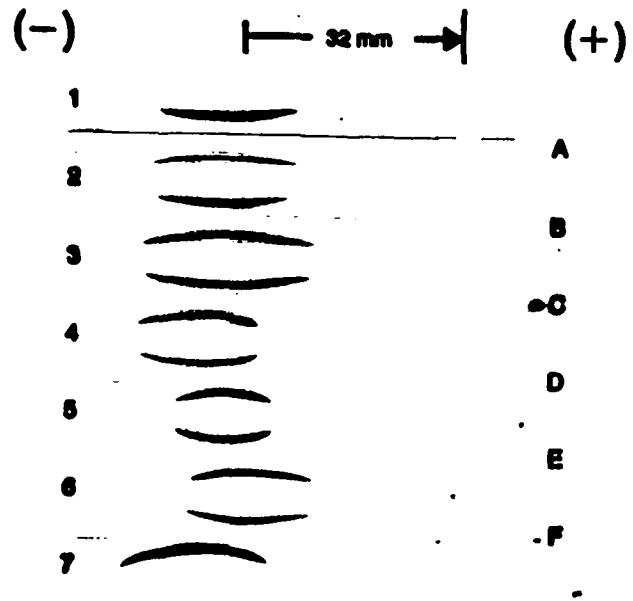
6 Post QAE pH 4.0 Elution

7 Final Product

Figure 22

Samples Electrophoresed For 120 Minutes At 120 Volts
Antisera: Cappel Anti Whole Monkey

Immunoelectrophoresis of Monkey ALF-2



1 Pre Batch QAE

2 Post Batch QAE

3 Pre QAE

4 Post QAE Pool 1

5 Post QAE Pool 2

6 Post QAE pH 4 Elution

7 Final Product

Figure 23

Samples Electrophoresed For 120 Minutes At 120 Volts
Antisera: Cappel Anti Monkey IgG $F(ab')_2$

Figure 24

FRACTIONATION FLOW SHEET

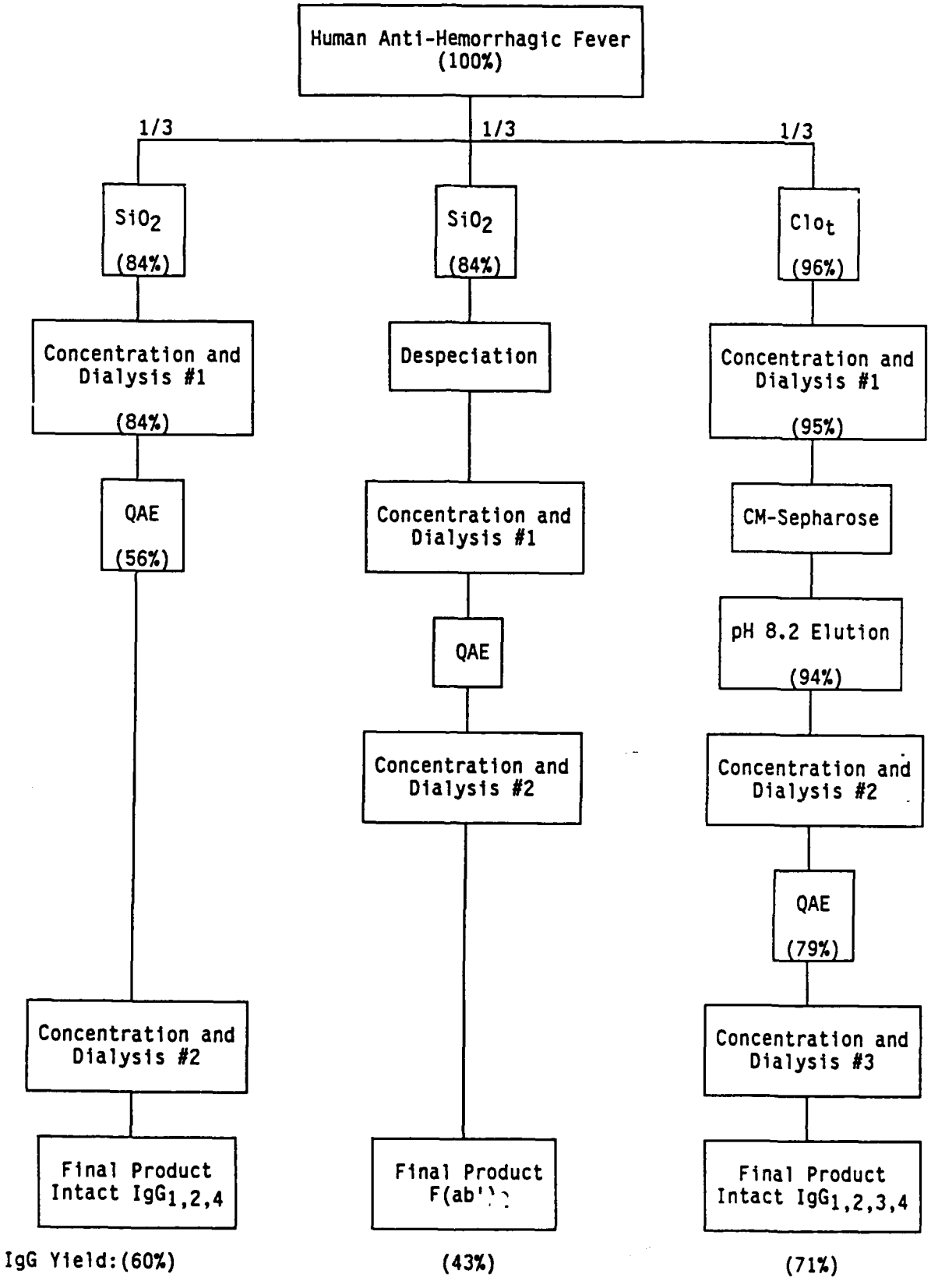
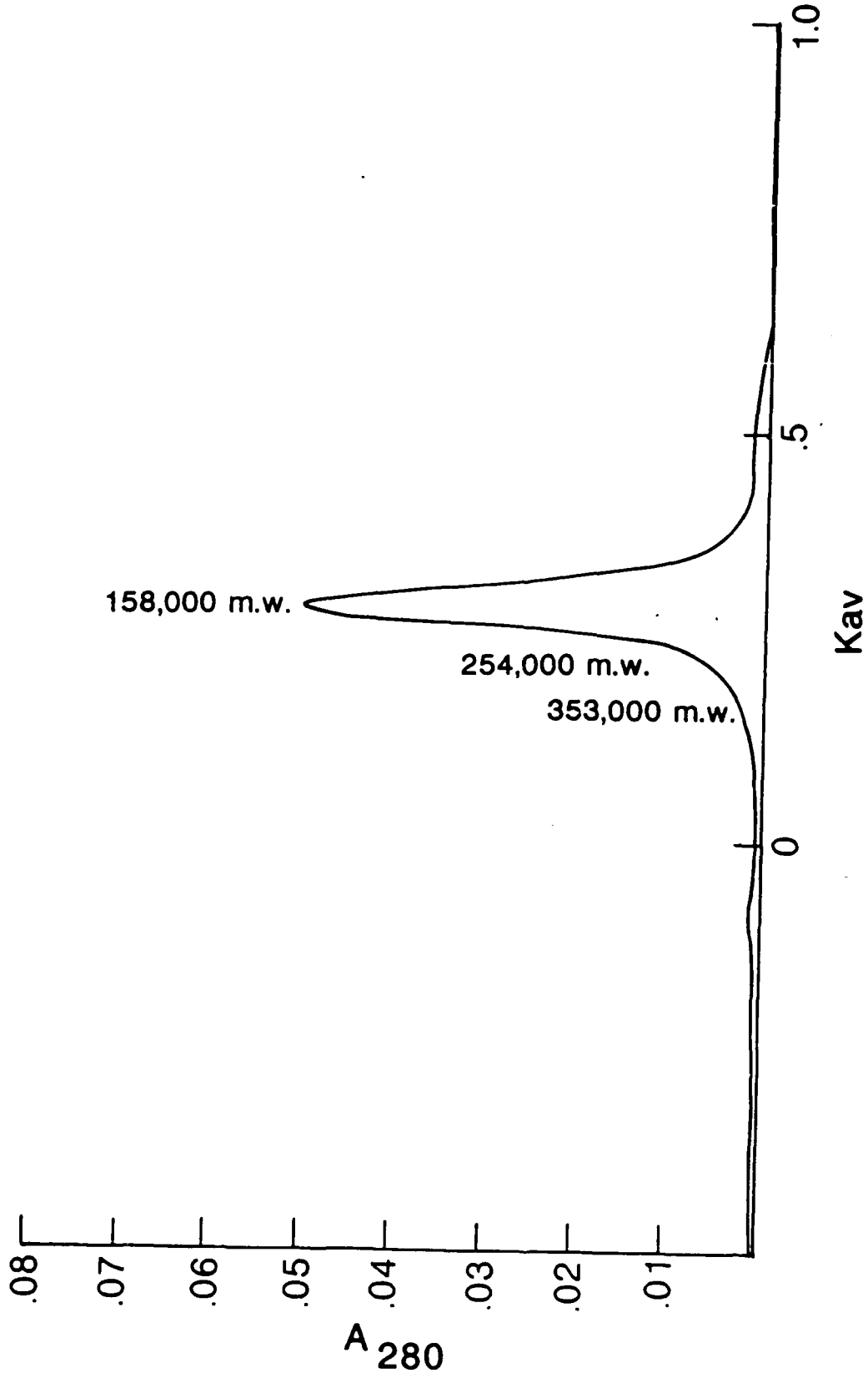
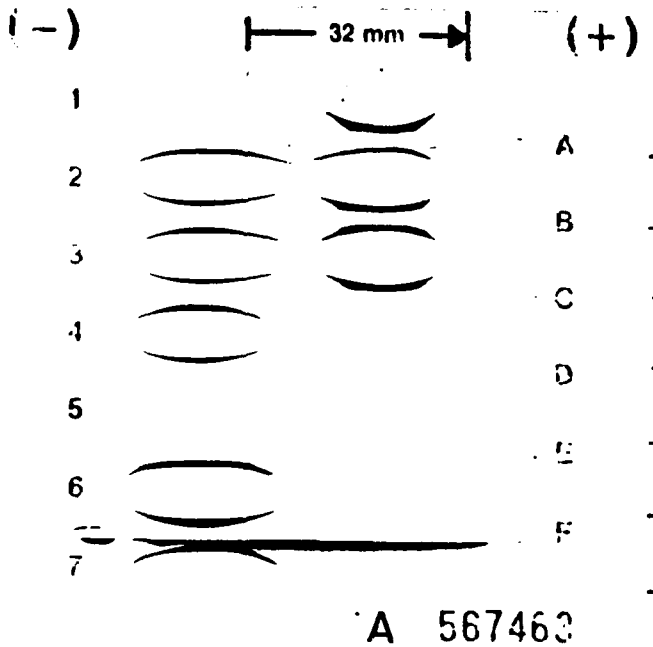


Figure 25

HPLC Human AHF-(CM) F.P. IgG



Immunoelectrophoresis of Human AHF -1(CM)



1 pH 5.0 Elution B

2 pH 8.2 Elution B

3 Pre QAE

4 Post QAE

5

6 Final Product

7 Final Product

Figure 26

Samples Electrophoresed For 120 Minutes At 120 Volts
Antisera: A-D,E;Rabbit Anti Whole Human,F;Rabbit Anti Human IgG

Figure 27

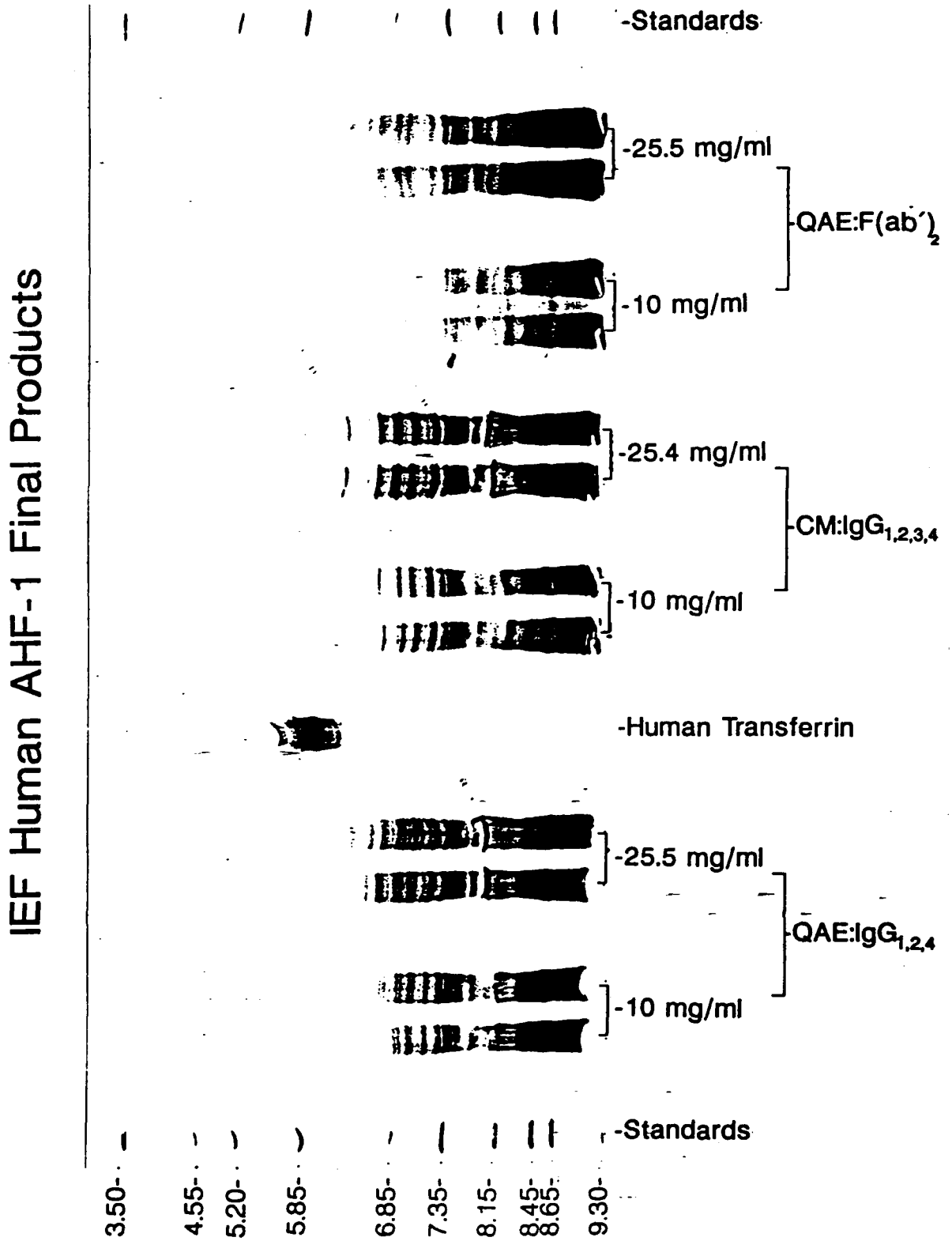
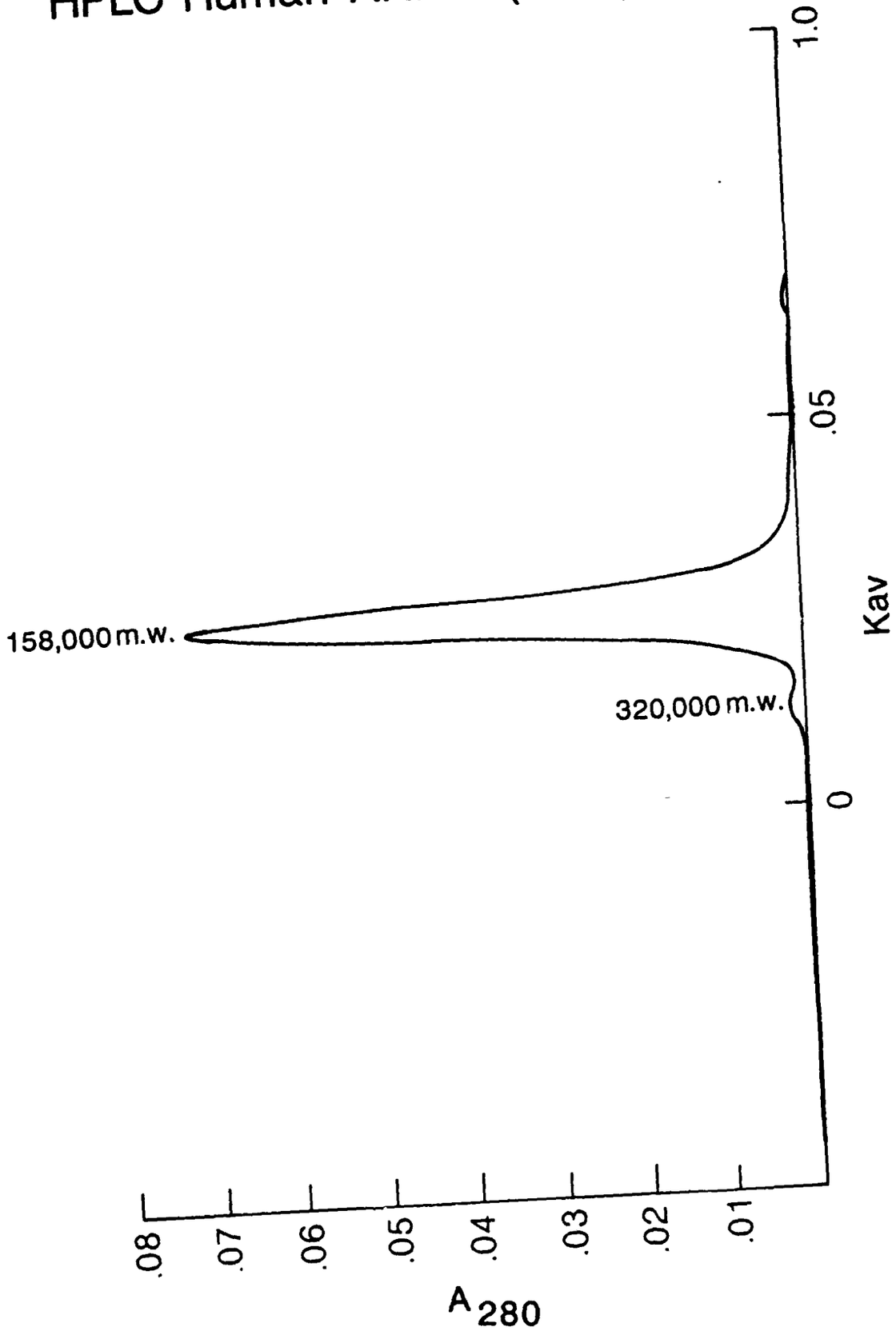
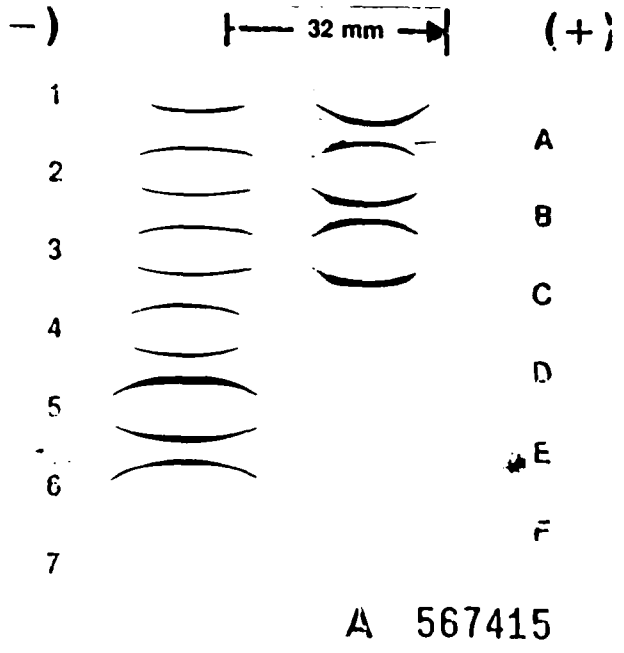

IEF Human AHF-1 Final Products
-Standards
-25.5 mg/ml
-QAE:F(ab')2
-10 mg/ml
-25.4 mg/ml
-CM:IgG1,2,3,4
-10 mg/ml
-Human Transferrin
-25.5 mg/ml
-QAE:IgG1,2,4
-10 mg/ml
-Standards
3.50-
4.55-
5.20-
5.85-
6.85-
7.35-
8.15-
8.45-
8.65-
9.30-

Figure 28

HPLC Human AHF-1 (QAE) F.P. IgG



Immunoelectrophoresis of Human AHF-1(QAE)



1 Plasma Pool

2 Post (A)

3 Pre QAE

4 Post QAE

5 Final Product

6 Final Product

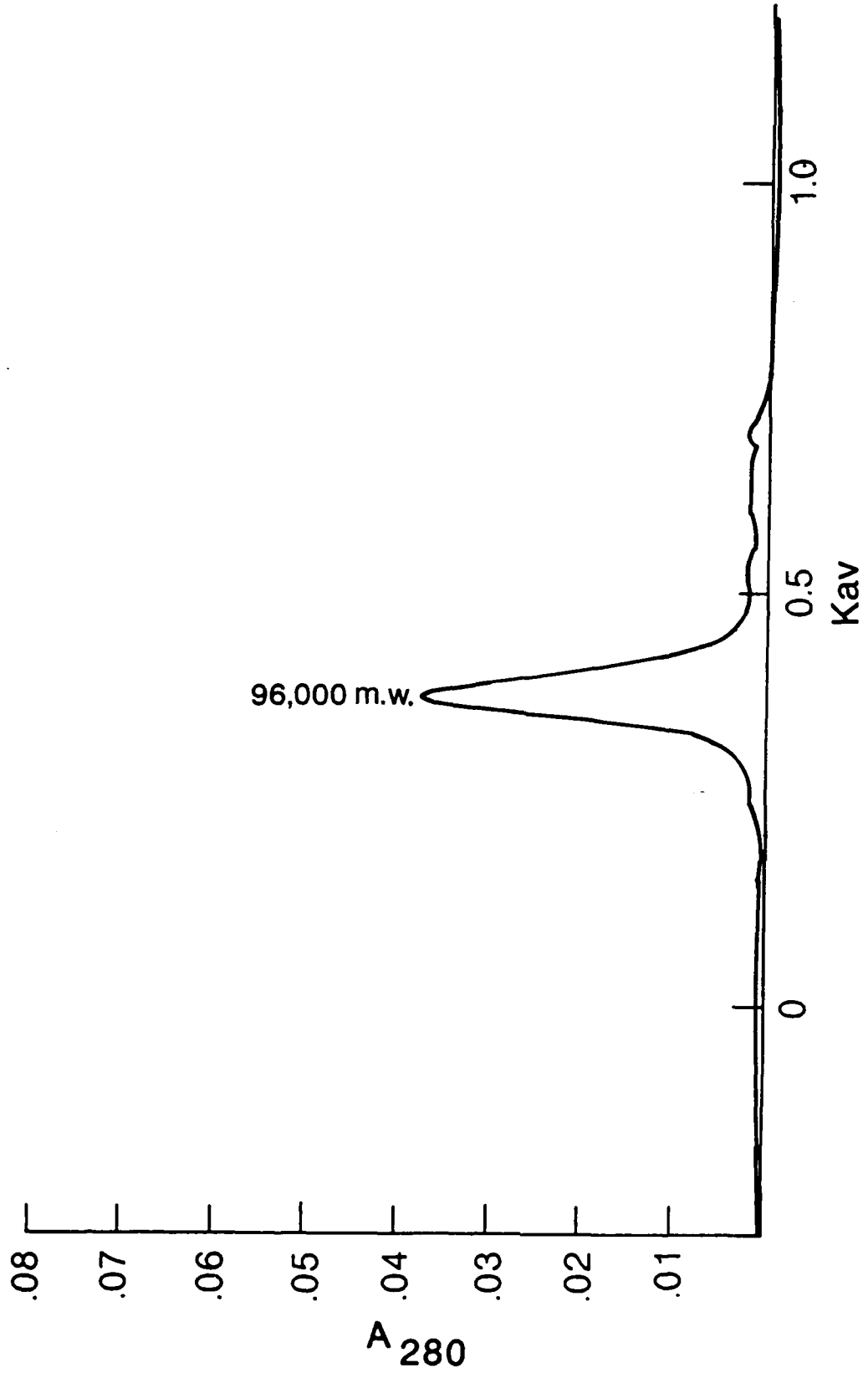
7

Samples Electrophoresed For 120 Minutes At 120 Volts
Antisera; A-D; Rabbit Anti Whole Human, E; Rabbit Anti Human IgG

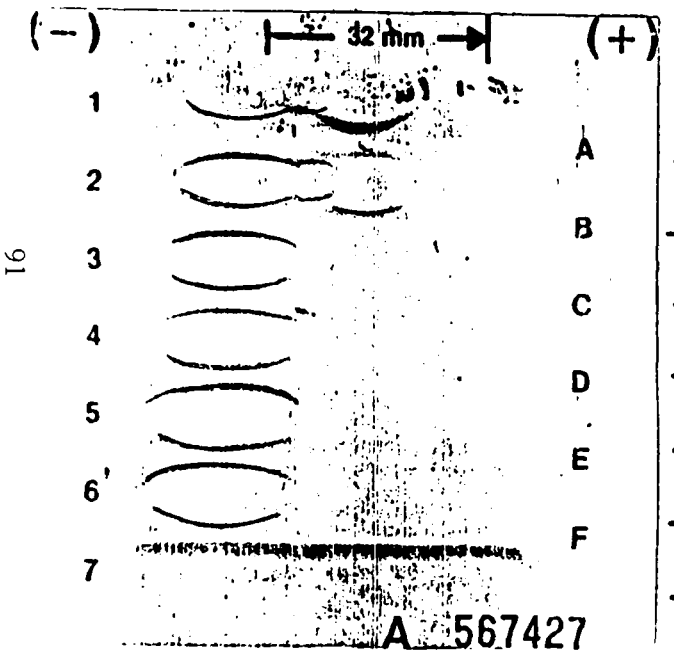
Figure 29

Figure 30

HPLC of Human AHF-1 $F(ab')_2$ F.P.



Immunoelectrophoresis of Human AHF-1 $F(ab')_2$



1 Plasma Pool

2 Post (A)

3 Post Despeciation

4 Pre QAE

5 Final Product

6 Final Product

7 Human $F(ab')_2$ Standard

Figure 31

Samples Electrophorsed For 120 Minutes At 120 Volts
Antisera: A-E; Rabbit Anti Whole Human, F; Rabbit Anti Human IgG

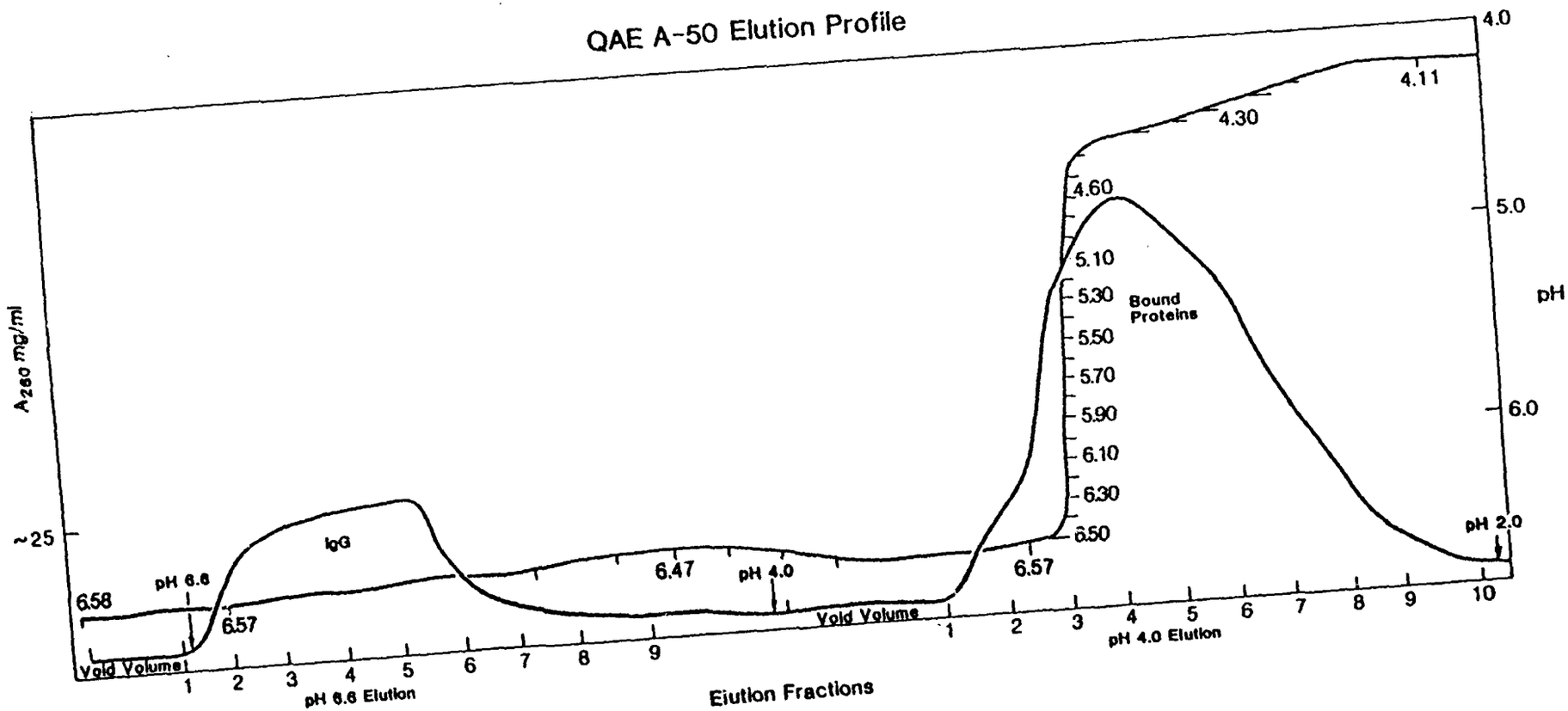

QAE A-50 Elution Profile
A280 mg/ml
~25
IgG
pH 6.6
6.58
6.57
Void Volume
1 2 3 4 5 6 7 8 9
pH 6.6 Elution
6.47
pH 4.0
Void Volume
1 2 3 4 5 6 7 8 9 10
pH 4.0 Elution
6.57
4.60
5.10
5.30
5.50
5.70
5.90
6.10
6.30
6.50
Bound Proteins
4.11
4.30
4.0
5.0
6.0
pH
pH 2.0
Eiution Fractions

Figure 32